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OTIS CHESTER WHIPPLE
1907–1941

J. C. Walker

One of the greatest shocks to a group of scientists is the sudden removal from their ranks by death of a young man on the threshold of a promising and useful career. Such was the case when news was received of the sudden collapse of Otis Chester Whipple at Kenosha, Wisconsin, while driving alone on the highway in connection with his research program in that area. This occurred on February 12, 1941; death followed a few hours later, early on February 13.

Whipple was born at Carrington, North Dakota, on February 18, 1907. He came from a long line of pioneers, one of whom had signed the Declaration of Independence. His parents had moved from Missouri shortly before his birth and when he was six years of age they went to California, where they are still engaged in fruit-growing near Sacramento. As a farm boy, Otis Whipple became thoroughly familiar with the practical aspects of agriculture, an experience that contributed to his pioneering but sound approach to applied plant pathology in later years. After graduation at Sacramento High School in 1926, he attended LaVerne College at Los Angeles for two years. He then returned to farming for another three years, attending Junior College at Sacramento for a year during that interval. He entered the University of California in 1931, where he received the Bachelor of Science degree in 1933.

During a year at Davis, California, under the guidance of Dr. J. B. Kendrick, he developed an interest in plant pathology that continued during his senior year and his first year of graduate study at Berkeley under Dr. M. W. Gardner. In the autumn of 1934 he transferred to the University of Wisconsin for further graduate studies leading to the Doctor's degree, which was granted in June, 1937. He was appointed to an instructorship in plant pathology at the University of Wisconsin in July, 1937, and held that position until his death. During this period he was in charge of the truck-crop-disease summer field laboratory near Kenosha in southeastern Wisconsin. On October 23, 1937, he was united in marriage with Miss Joan Young of Racine, Wisconsin, who survives him.

Whipple was a careful and original investigator. He showed these instincts early in his work with Dr. Gardner on spotted wilt of tomato and other plants. His researches at Wisconsin had to do primarily with diseases of the tomato and carrot, with several virus diseases of peas, beans, carrots, and cucumbers, and with spraying of potatoes, tomatoes, and other truck crops for disease control. At the time of his death he had won his spurs as a mature, independent investigator of unusual promise. He enjoyed the friendly respect and high regard of his colleagues, while outside the laboratory he quickly won the friendship and confidence of growers, canners, and other agriculturists with whom he came in contact.

OTIS CHESTER WHIPPLE
(1907–1941)

Publications in which Dr. Whipple was sole or joint author follow:

Spotted wilt of tomatoes and its transmission by thrips. (Abstract) Phytopath. **24**: 1136. 1934.

Spotted wilt of truck crops and ornamental plants. (Abstract) Phytopath. **25**: 17. 1935.

Spotted wilt of garden pea. Phytopath. **26**: 918–920. 1936.

Two strains of cucumber virus on pea and bean. (Abstract) Phytopath. **28**: 22. 1938.

Strains of cucumber mosaic virus pathogenic on bean and pea. Jour. Agr. Res. [U.S.] **62**: 27–60. 1941.

Three years of potato spraying in southeastern Wisconsin. Amer. Potato Jour. **18**: 254–261. 1941.

Injury to tomatoes by lightning. Phytopath. **31**: 1017–1022. 1941.

HETEROTHALLISM AND VARIABILITY IN VENTURIA PIRINA[1]

M. H. Langford and G. W. Keitt

(Accepted for publication July 28, 1941)

Knowledge of variability in the pathogen is a basic factor in the understanding of infectious diseases and their control. Recent contributions to the knowledge of reproduction in the fungi (*e.g.*, 3, 4, 6, 7, 10, 18, 22) have greatly facilitated genetic studies on phytopathogenic fungi. Demonstrations that new races of pathogenic fungi may arise by hybridization or mutation (24) have increased the knowledge of heritable variation in certain fungous pathogens. While extensive studies of variability and inheritance have been made on the rust and the smut fungi (24) and certain saprophytic Ascomycetes, especially *Neurospora* (*e.g.*, 7, 8, 9, 20), the parasitic Ascomycetes have received less attention. Keitt and Palmiter (17) and Keitt and Langford (16) have recently studied variability and inheritance in the apple (*Malus sylvestris* Mill.) scab pathogen, *Venturia inaequalis* (Cke.) Wint. The present paper reports similar work on the closely related fungus, *Venturia pirina* Aderh., the causal agent of pear (*Pyrus communis* L.) scab.

Wiesmann (26) showed that *Venturia inaequalis* and *V. pirina* are species comprising many biotypes that differ in pathogenicity and in morphologic and physiologic characters. His essential findings with reference to *V. pirina* were confirmed and extended by Herbst (13). Herbst, Rudloff, and Schmidt (14) state that, although valid proof is lacking, it may be assumed that the morphotypes of *V. pirina* and of several other species of *Venturia* are hereditary races in which combinations occur. Keitt and Palmiter (17) have reviewed literature relating to inheritance in *V. inaequalis* and have shown experimentally that combinations are a major source of heritable variations in that fungus.

The work reported herein has dealt with 5 sets of monoascosporic isolates of *V. pirina*, each set comprising 8 isolates from a single ascus, with record of the serial order of the spores. A brief preliminary report of this study has been published (19).

ISOLATION OF THE SETS OF 8 SPORES

In July, 1938, the 8 spores from each of 5 asci were isolated from Anjou pear leaves that had been collected in March near Hood River, Oregon, and forwarded to the writers by Mr. Leroy Childs. These leaves were kept air-dry at 4° C. until the isolations were made. The method of isolation has been reported elsewhere (16).

After all the spores from an ascus had been isolated and had germinated, each was transferred to a separate plate of malt agar. The isolates were

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

numbered in the order of occurrence of spores in the ascus, beginning with the apical one. The sets of 8 spores were designated A, B, C, D, and E in the order of their isolation. Each ascus was taken from a different perithecium.

CULTURAL CHARACTERS OF THE ISOLATES

Wiesmann (26) and Herbst (13) have shown that monosporic cultures of *Venturia pirina* grown *in vitro* vary greatly in morphologic and physiologic characters. The experiments reported below were performed to study the differences in cultural characters of the 8 isolates from each of several asci and to gain evidence as to the constancy of these characters through several successive monoconidial transfers.

Materials and Methods

The isolates were grown at 16° C. on malt agar (Trommer's malt extract, 2.5 per cent; agar, 1.7 per cent). When the ascosporic colonies were 12 days old, the conidial production, diameter, density, and type of growth of each colony were recorded, and a photomicrograph was taken. Then mycelial transfers and monoconidial transfers were made to malt-agar slants and plates, respectively. Throughout the investigation the mycelial transfers were carried independently of the monoconidial transfers described below.

From each of the 40 ascosporic colonies, 7 monoconidial transfers were made by the method described by Keitt and Langford (16), 3 to plates containing 10 cc. each of clear malt agar and 4 to plates containing 20 cc. After the former had been incubated 12 days, a record similar to that described for the ascosporic colonies was taken. The second group of plates was kept 8 weeks, then photographed. A record was taken of the conidial production, color, type of margin, and diameter of each colony.

Thereafter, the 16 isolates of sets C and E were carried in quadruplicate by monoconidial transfers. At the end of each 8-week period, the cultures were characterized as described above, and monoconidial transfers were made. The isolates were carried in this manner through 8 successive transfers. Photographs were then taken for comparison with those of the ascosporic colonies. All isolates were carried on malt agar slants at 16° C. by mycelial transfers at 8-week intervals.

Results

Differences of Isolates.—The 8 isolates of each set comprised 4 groups of 2 isolates each on the basis of colony characters. The 2 isolates of each pair were identical in colony characters, but distinguishable from all other pairs. Thus, among 40 isolates studied, 20 colony types could be distinguished. Figure 1 illustrates this for sets B, C, and E.

Each group of 2 isolates showing the same colony characters, hereinafter designated pairs of isolates, usually were derived from adjacent spores in the ascus. Thus, figure 1 shows that in sets B and C the pairs are 1–2, 3–4, 5–6, 7–8, while in set E they are 1–2, 3–5, 4–7, 6–8. The occurrence of pairs

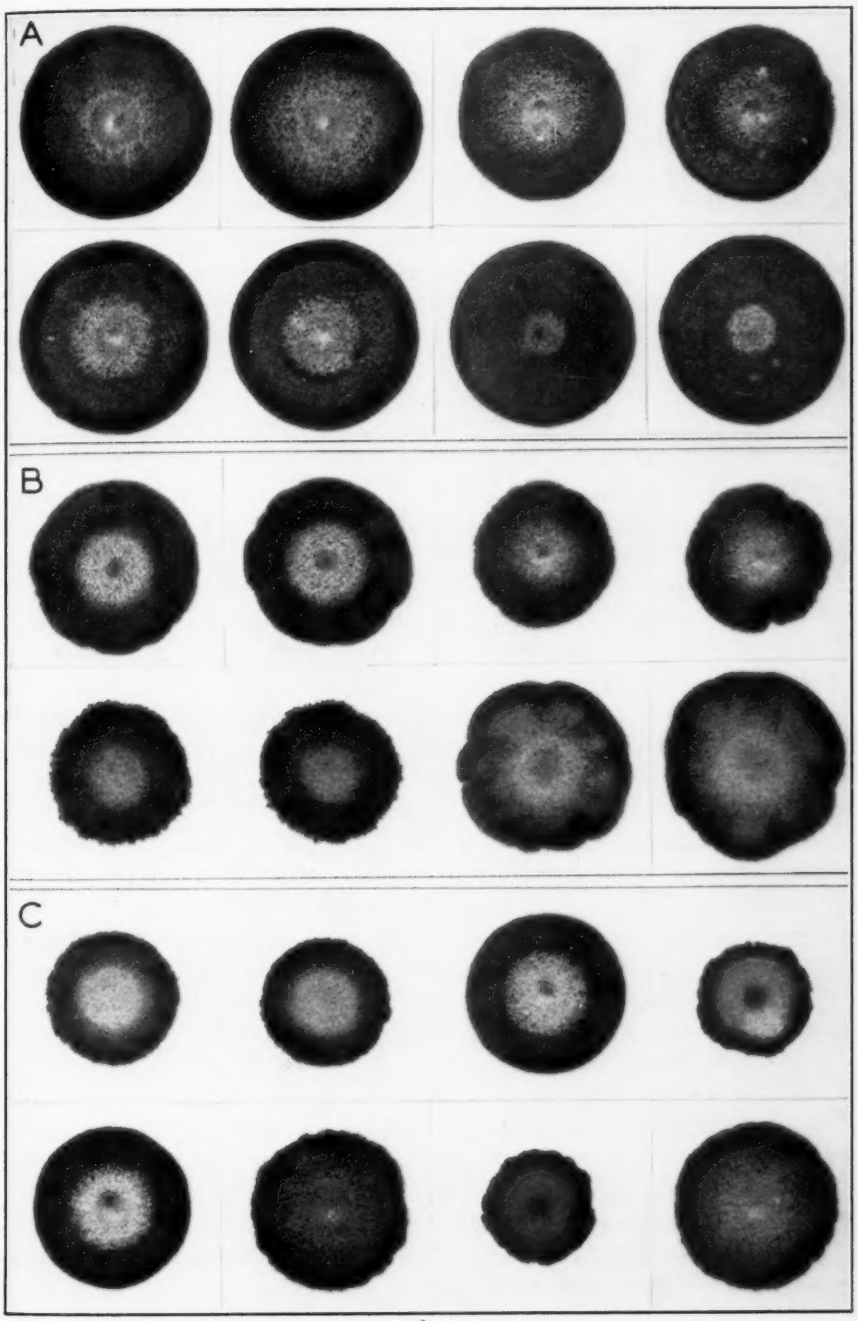

FIG. 1. Monoconidial colonies of *Venturia pirina* after 8 weeks on malt-agar plates at 16° C. The isolates in each set are numbered in the order of occurrence in the ascus of the 8 spores from which they were derived: upper row, left to right, 1, 2, 3, 4; lower row, 5, 6, 7, 8. × ½. A. Set B. B. Set C. C. Set E.

of isolates in set D is the same as that given for set E; in set A it is 1–2, 3–5, 4–6, 7–8.

Assuming that the third nuclear division in the ascus is equational, three possible explanations are offered for the failure in some cases of spores of like genetic constitution to lie next to each other in the ascus: (a) The spindle fibers may be crossed during the third nuclear division. For example, the spindle fibers of nuclei 1 and 2 may overlap those of nuclei 3 and 4, thereby putting nucleus 3 nearer the apex of the ascus than nucleus 2. (b) One nucleus may migrate beyond another before free spore formation begins. (c) Spores may change position. Thus, the arrangement in sets D and E could have resulted from spores (or nuclei) 4 and 5, also, 6 and 7, exchanging places. The arrangement in set A similarly could have resulted from exchange of position of spores 4 and 5.

The nature and extent of differences in colony characters among pairs of isolates within a set varied in the several sets. Thus, the isolates in set B (Fig. 1, A) differed little in growth rate, density, type of margin, or elevation of the central mass, whereas those in set E (Fig. 1, C) differed markedly in all these characters.

The basic color of the aerial mycelium of all isolates studied was brown. The shade of brown, however, varied greatly among the different pairs of isolates. Colonies were often zonate, the center usually being lighter than the outer zones (Fig. 1).

Certain pairs of isolates produced conidia more profusely than others. However, since sporulation was abundant in all cases, no attempt was made to classify the isolates on the basis of conidial production. Also, since the conidia produced by any isolate varied greatly in size, no attempt was made to determine differences in size of conidia produced by different isolates.

Constancy of Cultural Characters.—A comparison of A and B of figure 2 shows that the 8 isolates of set C had undergone little change in microscopic colony characters after passing through 8 successive monoconidial transfers. The macroscopic colony characters showed similar constancy. Set E, barring sectors, remained equally constant in culture. Of the 8 isolates in set C, 232 monoconidial plate cultures were grown for 8 weeks each during the period of 16 months covered by this study. No sectors appeared on any of these. On 220 cultures of set E, grown during the same period and under like conditions, one or more sectors appeared on each of 5 different isolates, a total of 8 sectors having been recorded. Thus, under the conditions of this experiment, the isolates of set E were less stable than those of set C.

Several sectors were isolated and grown under conditions similar to those described for the original isolates. Those that produced no conidia were propagated from hyphal tips. All sector lines had a higher growth rate, as indicated by colony diameter, and produced fewer conidia (none in some cases) than the isolates from which they sprang. They also differed from the original isolates in color, gray sectors frequently arising from brown

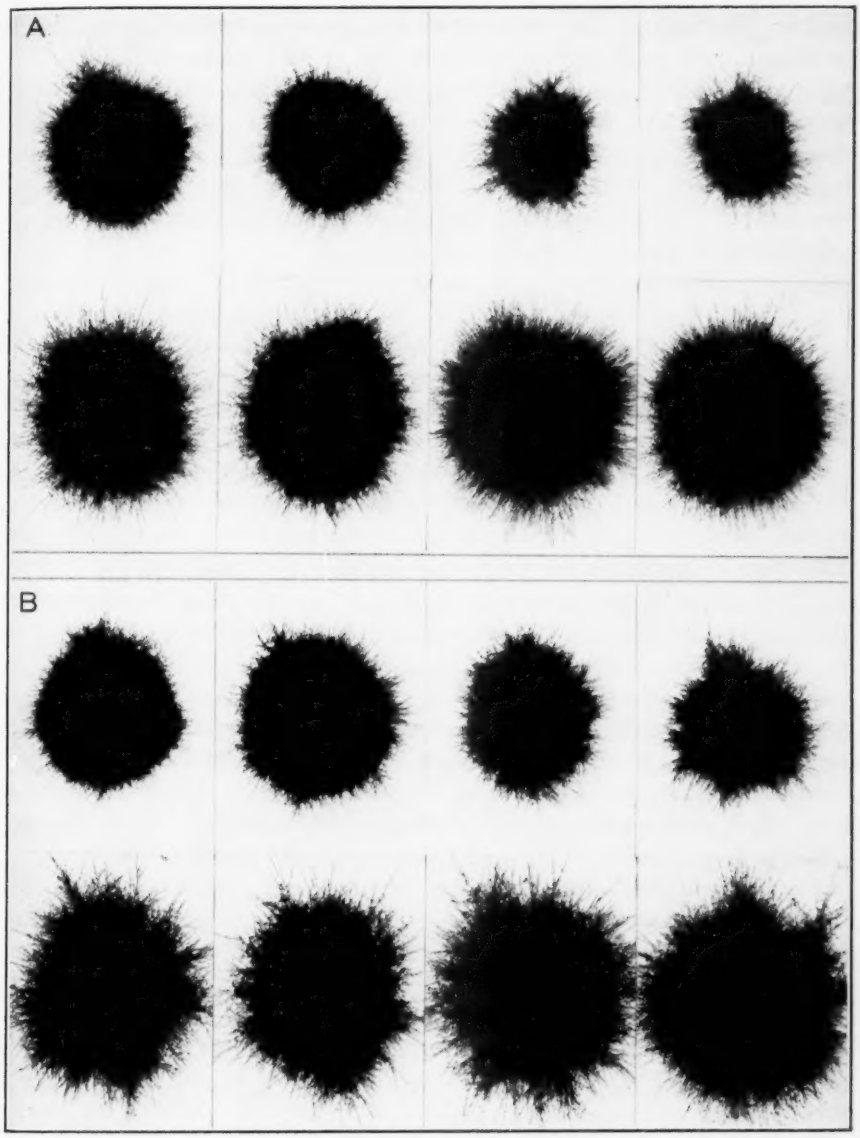

FIG. 2. Photomicrographs of monosporic colonies of the 8 isolates of set C, of *Venturia pirina*, after 12 days on malt-agar plates at 16° C. The isolates in each figure are numbered in the order of occurrence in the ascus of the 8 spores from which they were derived: upper row, left to right, 1, 2, 3, 4; lower row, 5, 6, 7, 8. A. Ascosporic colonies, photographed July, 1938. ×7. B. Colonies of the 8th monoconidial transfer, photographed October, 1939. ×6.

colonies. The colony characters of all sectors have been maintained through several transfers.

BREEDING THE FUNGUS IN VITRO

The writers know of no case in which the production of ascospores of *Venturia pirina* in culture has hitherto been reported. In order to determine

whether this fungus is heterothallic or homothallic, a method of producing the ascigerous stage *in vitro* was desired. In 1938, Keitt and Palmiter (17), working with the closely related fungus, *V. inaequalis*, reported that perithecia were produced *in vitro* from some compatible pairings, but not from others. Later, Keitt and Langford (16) developed a method by which mature perithecia of *V. inaequalis* are produced *in vitro* consistently and abundantly. This method, with modifications, was used in producing the perfect stage of *V. pirina* in plate cultures.

Materials and Methods

Preliminary Experiments.—Keitt and Langford (16) found that addition of a decoction of dead apple leaves to malt agar greatly improved it as a medium for growing the ascigerous stage of *V. inaequalis*. Similar use of a decoction of dead pear leaves in the present work gave like results with *V. pirina*. The decoction from leaves of a variety (Kieffer) resistant to the isolates studied was as effective as one from a highly susceptible variety (Anjou), whereas a decoction from McIntosh apple leaves did not stimulate production of perithecia of the pear-scab pathogen. Ash from dead pear leaves showed little or no stimulatory effect. The nature of the stimulatory substance has not been further investigated, but it would appear to be some thermostable organic component of the dead leaves.

Alfalfa stems, sterilized and placed on malt agar before it had solidified, gave a local stimulatory effect, which was, however, less marked than that produced by dead pear leaves. Perithecia were found in some cases partly imbedded in the stems. Pear twigs, used similarly, gave only a slight stimulatory effect, and in no case were perithecia found growing on, or imbedded in, the bark.

The Method Used.—A small piece of mycelium from each isolate was macerated in a separate flask of sterile water. For each pairing about 1 cc. of this suspension of spores and mycelium was poured from each of the 2 desired flasks into a sterile Petri dish. Then 20 cc. of malt agar (Trommer's malt extract, 0.5 per cent; agar, 2.5 per cent), in which a decoction of dead pear leaves had been incorporated, was added to each plate after having been cooled to about 42° C. In order to mix the 2 isolates well, the plate was rotated before the medium had solidified.

The leaf decoction was prepared from dead pear leaves that had been collected in September and stored air-dry in the laboratory. They were placed in a pan, covered with distilled water, and steamed in an Arnold sterilizer for 40 minutes. The decoction from 25 grams of these leaves was incorporated in each liter of medium. The medium was then sterilized at 15 pounds' pressure for 20 minutes.

Using the medium and the technique described above, each of the 40 isolates of the 5 sets was grown separately, and each was paired with every other isolate within its own set of 8. Also, each isolate in set A was paired with each one in set C; and likewise, set B was paired with set D.

Additional pairings were made on dead pear leaves in test tubes. In each tube a leaf was placed so that it adhered to the glass wall and dipped into a 0.5 per cent malt extract decoction, about 3 cc. of which was put into each tube. After sterilization, these leaves were smeared with the fungus. All possible pairings within each of the 5 sets of isolates were made in this manner.

Both the plate and the tube cultures were incubated at 20° C. for 12 days, then at 7° C. until the perithecia came to maturity. The higher temperature was used during the first 12 days to favor vegetative growth without too abundant formation of conidia. The lower temperature checked vegetative growth and favored the formation of perithecia.

Results

Perithecial initials were first observed in some of the cultures 26 days after the experiment was started, or 14 days after the temperature was lowered to 7° C. Ten days later initials were abundant in most cultures. An examination at this time showed that each isolate grown singly had produced both antheridia and ascogonia; thus the fungus is hermaphroditic. Several antheridia were appressed to the trichogyne of each perithecial initial observed. After the cultures had been incubated for 2 months, about half of the pairings had produced abundant perithecia, which were large enough to be visible to the naked eye. Asci were found in some of these. Three weeks later mature ascospores were first observed. An additional month was required to bring the perithecia in most pairings to the peak of maturity, while other pairings produced no mature ascospores until still another month had passed. Thus, some pairings produced ascospores in less than 3 months while others required almost 5 months. Since all plates were seeded at the same time and were grown under like conditions, this difference in time of maturity is thought to be due to a difference in the genetic constitution of the isolates. Attention is invited to the fact that after the first 12 days the plates were incubated at 7° C. It is likely that the time required

TABLE 1.—*Production in vitro of perithecia and ascospores of Venturia pirina from pairings of the 8 monoascosporic isolates of set B*[a]

Isolate number[b]	Isolate number							
	1	2	3	4	5	6	7	8
1	0	0	0	0	+	+	+	+
2		0	0	0	+	+	+	+
3			0	0	+	+	+	+
4				0	+	+	+	+
5					0	0	0	0
6						0	0	0
7							0	0
8								0

[a] 0 = No perithecia, initials only. + = Perithecia containing viable ascospores.
[b] The isolates are numbered in the order of occurrence of the spores in the ascus, beginning with the apical one.

for all pairings to produce mature perithecia would have been shortened by incubating the plates at a higher temperature after the early ascus stage was reached.

Table 1 shows that on the basis of sexual compatibility the 8 isolates of set B fall into 2 groups of 4 isolates each, which are intra-group incompatible and inter-group compatible. Similarly, the isolates of each other set can be divided into 2 groups. All isolates used in this study were found to be self-incompatible.

The results of all possible pairings of the isolates in set B with those in set D are shown in table 2. The 16 isolates of these 2 sets fall into 2 groups

TABLE 2.—*Production in vitro of perithecia and ascospores of Venturia pirina from pairings of the 8 monoascosporic isolates of set B with those of set D*[a]

Isolate number set B[b]	Isolate number, set D[b]							
	1	2	3	5	4	6	7	8
1	+	+	+	+	0	0	0	0
2	+	+	+	+	0	0	0	0
3	+	+	+	+	0	0	0	0
4	+	+	+	+	0	0	0	0
5	0	0	0	0	+	+	+	+
6	0	0	0	0	+	+	+	+
7	0	0	0	0	+	+	+	+
8	0	0	0	0	+	+	+	+

[a] 0 = No perithecia, initials only. + = Perithecia containing viable ascospores.
[b] See footnote b, table 1.

of 8 isolates each; isolates B 1, 2, 3, 4, and D 4, 6, 7, 8, composing one group, while B 5, 6, 7, 8, and D 1, 2, 3, 5, compose the other. The isolates of each group are intra-group incompatible and inter-group compatible. Pairings between sets A and C gave similar results. There has been no indication in this work of more than 2 groups for sexual compatibility.

It was mentioned earlier in this paper that some compatible pairings of isolates produced mature perithecia much earlier than others. Figure 3

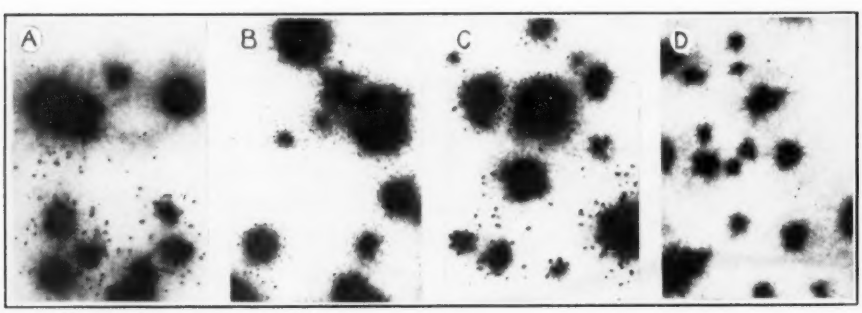

FIG. 3. Perithecia of *Venturia pirina* produced *in vitro* from fertile pairings of isolates, all of which were grown under similar conditions. ×2. A. C 1×C 3, showing large perithecia. B. C 1×C 8, showing small perithecia. C. E 3×E 8, showing abundant perithecia. D. E 7×E 8, showing sparse perithecia.

shows the variation in number and size of perithecia produced by different compatible pairings. The mature perithecia produced by C 1 × C 3 averaged about 225 μ in diameter, while those produced by C 1 × C 8 averaged no more than 75 μ. E 3 × E 8 produced at least 25 times as many mature perithecia as E 7 × E 8. Furthermore, a single perithecium of the former pairing contained several times as many asci and ascospores as did one of the latter.

The results given above were obtained from plate cultures containing malt agar in which a decoction of dead pear leaves had been incorporated. The cultures on sterilized pear leaves plus malt decoction in test tubes gave similar results. The latter medium, however, is much less satisfactory.

PATHOGENICITY OF THE ISOLATES ON 6 PEAR VARIETIES

In selecting pear varieties for pathogenicity tests with 2 sets of isolates, an attempt was made to use varieties representing different degrees of susceptibility as reported by most investigators (*e.g.*, 1, 2, 5, 11, 21, 23) in this country. Thus, Anjou, Flemish Beauty, Duchess, Seckel, Bartlett, and Kieffer were used. The first 4 varieties are generally listed among the more susceptible ones, while Kieffer is usually classed as highly resistant. Bartlett is considered relatively resistant by most American workers but is classed as highly susceptible in Australia (12, 25).

Materials and Methods

One-year-old pear trees were obtained from a nursery in midwinter. The tops were cut back to 18-inch whips, and the roots were trimmed to fit into 8-inch pots. After being potted the trees were kept in a basement at 10° C. for 12 days, then moved to a greenhouse where the temperature was approximately 16° C. By keeping all buds except 2 rubbed off, 2 vigorous shoots were obtained on each tree. After 8 or 10 leaves had developed on each shoot, the trees were inoculated by atomizing both surfaces of the leaves with a standardized suspension of conidia obtained from cheesecloth "wicks" adherent to the inner wall of 6-ounce bottles, each of which contained about 25 cc. of 2.5 per cent malt extract (Trommer's) solution (17).

In the latter part of March inoculations were made with each of the 16 isolates of sets C and E. One tree of each variety was inoculated with a given isolate. An uninoculated tree was included in each group as a control. The trees were kept in the moist chamber (15) at 16° C. for 48 hours, then returned to the greenhouse, where they were kept 4 weeks before results were taken.

After the results of the first inoculation had been recorded, one heavily infected leaf was picked from each of the Anjou trees, air-dried, and stored in a refrigerator at 4° C. The shoots on all trees were then cut back to about 2 inches and sprayed with Bordeaux mixture. From the buds in the leaf axils, 2 new shoots were allowed to develop on each tree. By June 1 these shoots had 6 to 10 leaves each. The experiment was repeated, inoculating the same trees with the same isolates that were used in the first inoculation.

One shoot on each tree was inoculated with conidia from culture and covered with a glassine bag, while the other shoot was inoculated with conidia of the same isolate from an Anjou leaf. During the day the greenhouse temperature usually rose somewhat above 16° C., but the similarity of the results to those obtained in the first experiment would indicate that this had little effect on disease development.

Results

Table 3 shows the results of inoculations on 6 pear varieties with the isolates of set C. The inoculations in March and those in June gave similar

TABLE 3.—*Results*[a] *from inoculating pear leaves in the greenhouse with the 8 monoascosporic isolates of Venturia pirina of set C*

Variety	Isolate number[b]							
	1	2	3	4	5	6	7	8
Anjou	++	++	++	++	++	++	++	++
Flemish Beauty	++	++	++	++	++	++	++	++
Duchess	+	+	−	−	+	+	−	−
Seckel	−	−	−	−	−	−	−	−
Bartlett	0	0	0	0	0	0	0	0
Kieffer	0	0	0	0	0	0	0	0

[a] ++ = Abundantly sporulating lesions. + = Moderately or sparsely sporulating lesions. − = Yellowish flecks (no sporulation). 0 = No macroscopic evidence of infection.
[b] See footnote b, table 1.

results in practically all cases. In the few instances in which any difference was observed, the higher values are given in the table. The inoculations with conidia from the host (Anjou leaves) and those from culture gave similar results in both number of lesions and type of infection.

The differences in varietal susceptibility to the 16 isolates tested are very striking. All isolates incited abundantly sporulating lesions on the leaves of both the Anjou and Flemish Beauty varieties, while none gave any macroscopic evidence of infection on either Bartlett or Kieffer. All isolates incited yellowish flecks but produced no conidia on Seckel leaves, and the 8 isolates of set E gave a similar result on Duchess. The 8 isolates of set C, however, comprised 2 groups of 4 isolates each as regards pathogenicity on the Duchess variety. Isolates 1, 2, 5, and 6 incited sporulating lesions, whereas isolates 3, 4, 7, and 8 gave only flecks. Inoculations with one sector line incited no infection on any variety, while another sector line gave lightly sporulating lesions on Anjou. No lesions or flecks developed on any of the control (uninoculated) trees.

DISCUSSION

On the basis of colony characters, each set of 8 isolates from a single ascus of *Venturia pirina* was found to comprise 4 distinct groups of 2 isolates each. The 2 members of each of these pairs of isolates, which were indistinguishable from each other by any test tried, were derived, with certain exceptions that have been discussed earlier in this paper, from spores that lay next to each

other in their order from apex to base of the ascus. It would appear, therefore, that, as in the case of *V. inaequalis* (16), the third nuclear division in the ascus is equational.

The constancy of colony characters and the maintenance of conidial production through 8 successive monoconidial transfers on malt-agar plates would indicate that the method employed has considerable merit for maintaining pure-line cultures. Since a sector of any considerable size can be seen easily on a plate culture, and since most sectors produce conidia sparsely or not at all, this method minimizes the possibility of unwittingly making the transfer from a sector, rather than from the original colony.

The breeding experiments show that *Venturia pirina* is heterothallic, in the sense that the 8 monosporic isolates from each ascus studied comprise 2 groups of 4 isolates each with reference to sexual compatibility, the isolates being hermaphroditic, self-incompatible, intra-group incompatible and inter-group compatible. Evidence from the serial order of the spores in the ascus in relation to the behavior of the isolates indicates that segregation for sexual compatibility may occur alternatively in the first or the second nuclear division in the ascus (*cf.* 16).

The differences among the several isolates in the quantity of conidia produced were not nearly so great as those among fertile pairings in the quantity of ascospores borne. Moreover, under similar conditions, certain pairings produced mature ascospores in 3 months, while others required 5 months. These differences, in both abundance and time of maturity of the ascospores, are thought to be traceable to differences in the genetic constitution of the isolates. It is well known that ascospores may be discharged from leaves in the field over a period of many weeks. Childs (5) reported that in the Hood River Valley of Oregon (the source of the isolates used in this study) ascospores may be discharged from February to September. Although other factors exert an influence, it is suggested that the difference in time required for different pairings of isolates to produce mature perithecia may be an important factor in prolonging the period of ascospore discharge in nature.

No variety of *Pyrus communis* is known to be immune from scab. A survey of the literature, however, shows a lack of agreement among investigators concerning the relative susceptibility of many pear varieties. In some cases, varieties that have been classed as resistant in certain countries or localities have been highly susceptible in others. Doubtless, differences in the environmental conditions and the consequent influence on host susceptibility offer a partial explanation for these discrepancies, but the available evidence indicates that variability in the pathogen is, also, a major factor. Herbst (13) reported the occurrence in some localities of forms of *Venturia pirina* capable of attacking varieties that in other localities remained free of infection. The isolates used in the present study gave only flecking without sporulation on the Seckel variety, which has been reported as highly susceptible by most American investigators (*e.g.*, 2, 11, 21); and no isolate gave any

macroscopic evidence of infection on Bartlett, which has been reported as highly susceptible in Australia (12, 25).

The results of the present study afford an experimental demonstration of segregation for pathogenicity in *Venturia pirina*. Isolates 1, 2, 5, and 6 of set C incited typical sporulating lesions on Duchess leaves, whereas 3, 4, 7, and 8 gave only flecks without sporulation. From the serial order of the spores in the ascus in relation to the behavior of these lines it is apparent that in this instance segregation of factors for pathogenicity occurred in the second nuclear division in the ascus.

The facts that the 8 isolates from an ascus comprise 2 groups of 4 isolates each with reference to sexual compatibility and pathogenicity, respectively, and 4 groups of 2 isolates each on the basis of colony characters, justify the conclusion that in *Venturia pirina*, as in *V. inaequalis* (17), both the antheridium and the ascogonium of a fertile pairing contribute hereditary materials to the ascospores, and that segregation of genetic factors precedes ascospore formation. Combinations, therefore, afford an explanation for much of the variation found in this pathogen.

SUMMARY

The 8 spores of each of 5 asci of *Venturia pirina* were isolated in the order of their occurrence in the ascus and grown *in vitro*.

The 40 isolates studied comprise 20 biotypes of 2 isolates each, 4 biotypes being obtained from each ascus. Apparently, the third nuclear division in the ascus is equational.

It was found that the ascigerous stage could be produced abundantly in culture by seeding Petri dishes with conidial suspensions of 2 sexually compatible isolates, adding to each plate 20 cc. of 0.5 per cent malt extract agar in which a decoction of dead pear leaves had been incorporated, and incubating under suitable conditions.

The 8 isolates of each set were grown singly and in all possible pairings of isolates in the same set. The isolates in certain sets were paired with those in other sets in all possible combinations. They were found to be hermaphroditic, but self-incompatible. The 8 isolates from each ascus comprise 2 groups of 4 isolates each, which are intra-group incompatible but inter-group compatible. Likewise, the pairings between sets revealed only 2 groups for sexual compatibility. Segregation of factors for sexual compatibility occurred alternatively in the first or the second nuclear division in the ascus.

Anjou and Flemish Beauty leaves were heavily infected by each of the 16 isolates composing the 2 sets of isolates tested. Kieffer and Bartlett were infected by none of these isolates. Yellowish flecks were incited, but no conidia produced, by all isolates of both sets on Seckel leaves, while all isolates of one set gave similar flecking on Duchess. In the other set, however, 4 isolates incited sporulating lesions on Duchess, while the other 4 incited only flecks.

It is concluded that *Venturia pirina* is a species consisting of many biotypes that differ in morphology and pathogenicity. The fungus is heterothallic, in the sense that the thalli are hermaphroditic but self-incompatible and comprise at least 2 groups that are intra-group incompatible and inter-group compatible. Combinations are, therefore, a major source of heritable variations in this pathogen.

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REDUCTION IN FUNGICIDAL VALUE OF COPPER COMPOUNDS BY ORGANIC MATERIALS

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INTRODUCTION

In modern plant-disease control practices by spraying and dusting, it is more the exception than the rule when a fungicide is used by itself. In orchard spraying a tank-load of spray often contains 4 different materials, while a vegetable dust may contain as many as 5. Each year the list of materials that may be added to fungicides becomes more extensive. These materials, other than insecticides and diluents, are generally classified as wetting, spreading, dispersing, flocculating, buffering, sticking, and "safening" agents. Many of these are organic, some being synthetic, while others are pulverized plant products. As the physical and chemical characteristics of these materials differ so widely, a study is being made of their possible action on the fungicidal value of copper compounds.

Several workers have studied certain aspects of the interaction of these materials but scant attention has been paid to their effect on the fungicidal value of copper compounds.

This paper presents the results of a laboratory investigation on the effect of several organic materials on the fungicidal value of copper compounds. While this work was in progress it was learned[3] that J. B. Skaptason and F. M. Blodgett, of Cornell University, had found that derris powder reduced the control of a fixed copper compound against late blight of potato on Long Island. Their work has recently been published (6).

MATERIALS

The materials used in this work were as follows:

Copper Compounds: copper sulphate, copper oxychloride, red cuprous oxide, yellow cuprous oxide, and Bordeaux mixture.

Organic Materials: derris powder (4 per cent rotenone), pyrethrum powder, soya flour, alfalfa meal, corn starch, and activated charcoal (Norite).

Other Materials: Cherokee clay.

METHODS

Considerable attention was paid, for reasons discussed below, to the development of testing methods. Two experimental designs were used in studying the effect of the organic materials: (a) the toxic 50 per cent deposit of copper

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was held constant and the deposit of organic material varied, and (b) the deposit of organic material was held constant and the deposit of copper varied. In the first design the effect of varying deposits of organic material on fungicidal value at the LD50 point was measured, whereas, in the second design, a measure was obtained of the increase in copper deposit necessary to give LD50 in the presence of unit deposit of organic material. The relative significance of these two designs is being discussed in detail in another paper (1).

Particular attention to method was not necessary to show that derris, for example, reduced the fungicidal value of a copper compound, but, when the effect of derris on a series of copper materials was studied, particular attention to method was necessary.

It seemed reasonable at first, in accord with most current fungicide research, to compare all the copper materials on the basis of equivalent metallic copper. The fact is, however, that copper spray materials vary widely in their fungicidal value per unit of copper. Apparently, the various materials release different amounts of toxic copper into the spore drop. In view of this, it was necessary to adopt a testing procedure that would permit using the materials on the basis of equivalent *toxic* copper rather than equivalent *metallic* copper, *i.e.*, to so use them that they would give equal spore inhibition. This was done by applying deposits to glass slides which should inhibit 50 per cent of the spores (LD50). By so doing, the effect of the organic materials on the copper compounds was determined at a definite point.

The materials were used in various ways: as sprays, as dusts, or the copper used as a spray and the organic material dusted on the sprayed surface while it was still wet. However, the general testing procedure, except where otherwise noted, was to use the copper materials as sprays and then dust on the organic material. This was found to be the best procedure as the large particle size of some of the organic materials clogged the nozzle of the laboratory sprayer.

The sprayer used was a horizontal precision sprayer (4). The dusting apparatus consisted of a settling tower, a chamber for exposing slides at the base of the tower, and a system for introducing known charges of dust into the tower in uniform time (3). With this apparatus, the deposit of dust can be regulated.

The biological assay method recently described (4) was used to measure the effect of the organic materials on the fungicidal value of the copper compounds. The test fungus was *Macrosporium sarcinaeforme* Cav.

EXPERIMENTATION

As preliminary tests indicated that ground derris root caused reduction in fungicidal value of copper compounds, this material was used as the test organic material.

Effect of Derris on the Same Copper Compound Used as a Spray and as a Dust

Tests were made on the effect of derris on red copper oxide used as a dust and as a spray.

Dust Test. Two dusts were prepared, namely: red copper oxide-Cherokee clay (7–93) and red copper oxide-derris-Cherokee clay (7–18¾–74¼). A series of coated glass slides was dusted with each dust in such a way as to give a series of deposits. The fungicidal value was then determined. The data (Table 1) show that derris reduced the fungicidal value of the copper, that derris by itself had no effect on the test fungus, and that Cherokee clay had no effect on either the copper or the test fungus.

Spray Test. Red cuprous oxide was used alone and combined with derris in the same ratio as used in the dust. Slides were sprayed with each to give a deposit of metallic copper that should inhibit approximately 50 per cent of the spores (LD50). The data (Table 1) show that the derris reduced the fungicidal value of the copper.

TABLE 1.—*Effect of derris on the fungicidal value of a copper compound used as a spray and as a dust*

Material	Metallic Cu deposit	Derris deposit	Spore inhibition
	Gamma/cm.²	*Gamma/cm.²*	*Per cent*
Red cuprous oxide-Cherokee clay (7–93) (dust)	9.1	None	46
	4.5	"	29
	3.1	"	18
	2.6	"	15
Red cuprous oxide-derris-Cherokee clay (7–18¾–74¼) (dust)	7.1	21.4	6
	4.7	18.8	3
	3.5	10.6	1
Red cuprous oxide (spray)	6.0	None	45
Red cuprous oxide-derris (spray)	6.0	18.1	6
Derris (spray)	.....	24.0	1
Cherokee clay (spray)[a]	.....	.....	1
Check	.....	.....	1

[a] Deposit, 72 gamma/cm.²

The data in table 1 show that derris affects a copper compound alike as a dust or spray.

Effect of Derris on Different Copper Compounds

The tests were made as follows: five slides each were sprayed with Bordeaux and copper oxychloride to give LD50 deposits. One slide for each was held as it was, while the other 4 were dusted while still wet with different charges of derris to give 4 different deposits. The data for a typical test (Table 2) show that equal quantities of derris affected both copper compounds essentially alike at the LD50 point.

TABLE 2.—*Effect of equal quantities of derris on the fungicidal value of Bordeaux mixture and copper oxychloride at the LD50 point*

Copper compound	Derris deposit	Spore inhibition
	Gamma/cm.²	*Per cent*
Bordeaux mixture (metallic Cu deposit, 0.15 gamma/cm.²)	None	49
	1.3	51
	2.6	48
	5.1	57
	10.2	31
Copper oxychloride (metallic Cu deposit, 0.30 gamma/cm.²)	None	52
	1.3	57
	2.6	50
	5.1	49
	10.2	28
Check	.....	2

A test was made also using copper sulphate, a soluble copper compound, in the same maner as described above. The data (Table 3) show that there is a direct correlation between the amount of derris and the reduction in fungicidal value of copper sulphate—the more the derris the greater the reduction.

TABLE 3.—*Effect of amount of derris on the fungicidal value of copper sulphate*

Material	Derris deposit	Spore inhibition
	Gamma/cm.²	*Per cent*
Copper sulphate (metallic Cu deposit, 0.19 gamma/cm.²)	None	70
	2.8	55
	5.6	28
	11.2	13
	22.4	0
Check	.....	0

From these tests it seems probable that the deleterious effect of powdered derris root on copper fungicides is general as it affected Bordeaux mixture, copper sulphate, copper oxychloride, and cuprous oxide.

Effect of Different Organic Materials on Copper Compounds

In these tests diverse organic materials were used, namely: soya flour (a common supplement); derris powder, pyrethrum powder, and derris extract (insecticides); activated charcoal (a sorbent material); and alfalfa meal (a pulverized plant material not used in sprays or dusts).

Alfalfa Meal, Soya Flour, Derris. The effect of these materials on the fungicidal value of copper oxychloride was determined. The data (Table 4) show that all 3 materials reduced the fungicidal value, soya flour reducing it the most.

Pyrethrum, Derris, and Derris Extract (Rotenone). The effect of these

TABLE 4.—*Effect of alfalfa meal, soya flour, and derris on the fungicidal value of copper oxychloride*

Organic material	Deposit of organic material	Copper oxychloride	
		Metallic Cu deposit	Spore inhibition
	Gamma/cm.²	*Gamma/cm.²*	*Per cent*
Alfalfa meal[a]	None	0.32	58
	3.0	"	38
	6.0	"	35
	12.0	"	29
Soya flour[b]	None	0.32	58
	2.1	"	63
	4.2	"	52
	8.4	"	12
Derris[a]	None	0.32	58
	2.6	"	50
	5.2	"	49
	10.4	"	28
Check	.....	.....	2

[a] Had no effect on the test fungus.
[b] Stimulated germ tube development somewhat.

materials on yellow cuprous oxide was determined. The test was made in a different fashion.

Stock suspensions were prepared of yellow cuprous oxide (1½–100) and of the derris and pyrethrum powders (4–100); stock solutions (1–200, 1–400, and 1–800) of derris extract were prepared also. These stocks represent the concentration at which these materials are used in the field. They were diluted proportionately to strengths at which they could be used in the

TABLE 5.—*Amount of metallic copper deposit, using yellow cuprous oxide, required for LD50 in the presence and absence of various organic materials*

Material	Metallic Cu deposit required for LD50
	Gamma/cm.²
Yellow cuprous oxide	0.69
Yellow cuprous oxide + derris	1.08
Yellow cuprous oxide + pyrethrum	1.09
Yellow cuprous oxide + derris extract	
(1–800)	0.70
(1–400)	0.65
(1–200)	0.66

laboratory. A series of glass slides was so sprayed with each combination as to give a range of deposits; at each deposit the ratio of added material to copper was the same. The data on spore inhibition were plotted on logarithmic probability paper, and the amount of copper deposit necessary to give LD50 for the various combinations was determined (Table 5).

The data in table 5 show the following: when either derris or pyrethrum

powder was added to yellow cuprous oxide, 57 per cent more metallic copper was necessary to give LD50 than when the copper was used alone; derris extract (rotenone) had no effect on the fungicidal value; the rotenone in the derris powder is not the material responsible for its effect on copper compounds.

Activated Charcoal (Norite). This material was used, as it is highly adsorptive. Its effect on copper oxychloride was determined. Data obtained (Table 6) show that it had no effect on the fungicidal value of copper oxychloride.

TABLE 6.—*Effect of activated charcoal on the fungicidal value of copper oxychloride*

Copper compound	Activated charcoal deposit	Spore inhibition
	Gamma/cm.²	*Per cent*
Copper oxychloride (metallic Cu deposit, 0.50 gamma/cm.²)	None	62
	5.9	63
	15.5	63
Check	.....	0

Effect of the Nature of the Organic Material

The data for activated charcoal indicated the reduction in fungicidal value of copper compounds by organic powders probably was not caused by adsorption of soluble copper from solution, while that obtained comparing derris and derris extract (rotenone) indicated that it might be associated with the nature of the organic material. Accordingly, an attempt was made to determine if the nature of the organic material was related to the reduction. Three materials were selected—soya flour (high protein), corn starch (high starch), and activated charcoal (carbon)—and their effect on the fungicidal value of copper oxychloride determined.

Data obtained in a typical test (Table 7) show that the only material to reduce the spore-inhibiting power of copper oxychloride was soya flour,

TABLE 7.—*Effect of nature of the organic material on the fungicidal value of copper oxychloride*

Organic material	Deposit of organic material	Copper oxychloride	
		Metallic Cu deposit	Spore inhibition
	Gamma/cm.²	*Gamma/cm.²*	*Per cent*
Activated charcoal	None	0.41	49
	13.0	0.41	53
Corn starch	None	0.41	49
	13.0	0.41	49
Soya flour	None	0.41	50
	13.0	0.41	33
Check	.....	.....	1

the material of high protein content. Similar results were obtained also when Bordeaux mixture and red cuprous oxide were used in place of copper oxychloride.

These data suggest the possibility that the reduction in spore inhibiting powers of copper compounds by certain organic materials may be brought about by a reaction between soluble copper and the protein in the organic material.

Various organic materials used in this work were submitted to E. M. Bailey, Chief, Dept. of Analytical Chemistry, Connecticut Agricultural Experiment Station, for protein analysis. The analysis by the Kjeldahl gave for each kind of material the following percentages of protein ($N \times 6.25$) : Soya flour, 52.06; alfalfa meal, 13.00; pyrethrum, 11.63; derris, 9.13; corn starch, 0.56.

All of these materials, except corn starch, the material of lowest protein content, reduced the spore inhibiting powers of copper compounds. Further, the spore inhibiting powers of copper oxychloride were reduced the most by soya flour, the material of highest protein content.

DISCUSSION

Although the precise mechanism by which copper inhibits spore germination is not fully understood, it has been somewhat generally accepted that the fungicidal value of copper compounds is related to their solubility—the more soluble the material the higher its fungicidal value. It has been shown by Marsh (5), however, that certain soluble copper compounds, *e.g.*, cupric phthalocyanine, are not toxic; and Goldsworthy and Green (2) have presented evidence showing that the fungicidal value is not directly related to the solubility, but is related to the amount of "ionic" (available) copper produced by the material in solution. Irrespective of the nature of the *toxic* copper the same amount is present at the LD50 point, for example, for all copper materials. Thus, when an added material reduces the fungicidal value it must do so by reducing the amount of *toxic* copper present in solution. This may be done by precipitation of the copper from solution, by adsorption or adsorption of the toxic copper, or by chemical reaction between the toxic copper and the added material.

The deleterious action of organic materials on antiseptics is well known in the field of bacteriology. In relation to fungicides, Goldsworthy and Green (2) have pointed out that organic materials, such as those usually found in ordinary nutrient media, are capable of inactivating considerable quantities of soluble and ionic copper by adsorption.

The fact that activated charcoal did not reduce the fungicidal value of a fixed copper compound indicates that adsorption may not be the explanation for the reduction obtained with the various organic materials in this work.

Data obtained in this investigation indicate that the reduction in fungi-

cidal value of copper compounds by certain organic materials may be brought about by a reaction between the toxic copper and the protein in the organic material. This hypothesis is supported by data showing that materials low in protein did not cause reduction, whereas those having considerable protein did.

The laboratory data presented in this paper on red cuprous oxide are supported by field data secured by Skaptason and Blodgett (6) showing that the addition of derris or pyrethrum, alone or in combination, to this material reduced its control of late blight of potato. However, the use of laboratory data on the effect of organic materials on various copper compounds to forecast what will happen in the field must wait, for reasons discussed below, until field data on several copper compounds are available.

There are two factors involved in the protective value (disease controlling powers) of a copper compound in the field—fungicidal value and tenacity. Unpublished data secured by the writers over a period of years indicate that the protective value of a copper compound is more dependent on tenacity than on fungicidal value. For example, the protective value of red cuprous oxide, a material of high tenacity but comparatively low fungicidal value, is equal to that of copper oxychloride, a material of low tenacity but comparatively high fungicidal value. In the laboratory work reported here the influence of tenacity on the spore-inhibiting powers over a period of time has not been determined. Thus, field tests comparing the effect of organic materials on copper compounds of high fungicidal value and low tenacity, and vice versa, are required to determine the influence of tenacity. Such tests are being made.

Data presented showing that derris extract (rotenone) did not reduce the fungicidal value of a fixed copper compound suggest the possibility that the deleterious effects of derris and pyrethrum powders may be overcome by extracting the insecticidal fractions and impregnating them on a suitable inorganic or non-protein organic diluent for use in dust form.

SUMMARY

A laboratory study has been made of the effect of diverse organic materials on the fungicidal value of copper compounds. The copper compounds used were copper sulphate, Bordeaux mixture, copper oxychloride, and red and yellow cuprous oxide.

Materials containing considerable amounts of protein, *e.g.*, derris powder, pyrethrum powder, soya flour, and alfalfa meal, reduced the fungicidal value. The copper compounds were all affected similarly at a definite point (LD50).

Materials containing little or no protein, *e.g.*, activated charcoal and corn starch, did not reduce fungicidal value.

The reduction in fungicidal value by materials containing protein is

most likely brought about by a reaction between the toxic copper and the protein, thus reducing the amount of toxic copper available to the spores.

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RELATION OF MOISTURE TO INVASION OF TOBACCO LEAVES BY BACTERIUM TABACUM AND BACTERIUM ANGULATUM[1]

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In connection with a study on the method of infection of tobacco leaves by *Bacterium tabacum* Wolf and Foster and by *B. angulatum* Fromme and Murray, previous papers from this laboratory have reported that: (a) tobacco leaves atomized with a suspension of *B. tabacum* become infected only if stomata are open at the time of inoculation (5); (b) there is a correlation between stomatal opening and susceptibility of leaves to water-soaking; leaves can be water-soaked by a stream of water beating against the lower surface only when stomata are open (6); (c) leaves may be injured when kept water-soaked for a period of 24 to 48 hours (13).

The object of the experiments reported in this paper is to determine whether bacteria present in water on the surface of a tobacco leaf can invade the leaf in the absence of water-soaked tissue.

LITERATURE REVIEW

Clayton (3) has reported that water-soaking of tobacco leaves during rainstorms accompanied by wind facilitated invasion by the wildfire organism (*Bacterium tabacum*). Braun and Johnson (1) later found a close relation between the amount of water-soaking and the extent of natural infection by *B. angulatum* in tobacco seed beds.

Clinton and McCormick (4) reported that wildfire infection was not obtained by pouring a suspension of the bacteria onto leaves unless conditions were similar to those necessary for natural infection; but the conditions were not described. Riker (11) concluded that a great many more lesions developed on leaves when plants were placed in a moist chamber before and after inoculation than when placed in the moist chamber only after inoculation. But it has been known for years that wildfire and angular-leaf-spot infection can be produced by forcefully atomizing tender leaves of vigorous plants with a bacterial suspension, without placing the plants in a moist chamber before or after inoculation. The stomata, however, must be open at the time of inoculation, and the atomizer must be held close to the leaf surface; apparently the bacteria are then forced into the leaf through the stomata. Very little, if any, infection occurs if the atomizer is held more than 10 or 12 inches from the leaf surface.

There seems to be no complete agreement regarding the method of invasion of susceptible leaves by pathogenic bacteria. Smith (12) first pointed out the importance of keeping plants moist before spraying them

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

with bacteria to produce stomatal infection. McLean and Lee (10) have shown that citrus canker bacteria can enter leaves through stomata only when the stomatal pores are filled with water. Zaumeyer (14) found that infection of bean leaves by *Bacterium phaseoli* was most severe when plants were kept moist before inoculation. He suggested that this caused substomatal cavities to become well supplied with water, offering the bacteria a free swimming channel from the droplets of moisture on the surface to the interior of the leaf. Leach (9) recently has stated that a film of water connecting the surface of the epidermis with the substomatal chamber appears necessary for stomatal infection by bacteria. Heald (8), on the other hand, visualizes the method of bacterial invasion in the following way: "Suppose a single bacterium of a species pathogenic for the host finds lodgment in a film of water over a stomatal opening. Under favorable conditions cell division follows in rapid succession. The resulting bacteria are pushed or work their way into the stomatal opening and soon come to fill the substomatal chamber."

MATERIALS AND METHODS

Inoculations were made by pouring a suspension of bacteria onto the lower surface of leaves of vigorously growing Burley tobacco plants in greenhouse and field. Twenty-four-hour beef-peptone-broth cultures of *Bacterium tabacum* and *B. angulatum* were used. Just before inoculation the broth cultures were diluted with 50 parts of sterile tap water. To determine whether the leaves were physiologically susceptible to infection under the tested conditions similar leaves were inoculated by atomizing the bacterial suspension onto the lower surface with a deVilbis No. 15 atomizer, held 2 inches from the leaf, thus forcing bacteria into the leaves.

To water-soak leaves, a stream of water was forced from a 10-cc. B–D Champion hypodermic syringe against the leaf surface. The end of the needle of the syringe was held about 1 inch from the leaf surface. The stream of water was moved as rapidly as tissue became water-soaked. Unless stated otherwise, the stream of water was directed onto the lower surface. The water-soaked condition disappeared within 10 to 30 minutes.

RELATION OF SURFACE MOISTURE TO INFECTION

To determine whether bacteria present on leaf surfaces produce infection if the leaves are kept moist several hours after inoculation, the following experiments were performed.

Stomata Open. Leaves Moist After Inoculation

At 11:00 a.m. May 2, 1939, 4 greenhouse plants were inoculated by pouring a bacterial suspension onto the lower leaf surfaces. Stomata were open. Two of the plants were kept under bell jars 24 hours following inoculation, and the other 2 were kept in the greenhouse atmosphere. Seven

days later these 4 plants showed only a few isolated wildfire spots, whereas leaves of 4 similar plants, atomized with the bacterial suspension, were heavily infected. The experiment was repeated 3 times with the same results. Similar results were observed on field-grown plants. In some instances the bacterial suspension was examined microscopically before inoculation, and bacteria were seen to be motile.

Stomata Closed. Leaves Moist Until After Stomata Open

Diachun (5) has reported that tobacco leaves atomized with *Bacterium tabacum* became infected only if the stomata were open at the time of inoculation. The following experiments were conducted to determine whether infection occurs on leaves inoculated when the stomata are closed and then kept moist until the stomata open. The bacteria in the water on the leaf surface then would have access to open stomata. On April 18, 1939, at 8 p.m. 4 small plants were inoculated with the atomizer held 2 inches from the lower leaf surface. Most of the stomata were closed. Two of the plants were placed under bell jars to keep the leaf surfaces moist until the stomata would open the following morning. The other two were left in the greenhouse atmosphere to permit the leaf surfaces to dry. The leaves of the plants under the bell jars were still moist at noon the next day, when the plants were removed from the moist air and placed in the greenhouse. Six days after inoculation only a very few wildfire spots were present. There was no more infection on the leaves that had been kept moist until the stomata opened than on those allowed to become dry. But numerous wildfire spots covered the leaves of similar plants inoculated in the same way at 11 a.m., when the stomata were open. This experiment was repeated 3 times, with the same results. Similar results were obtained in the field.

Leaves Moist Before and After Inoculation

Riker (11) found that tobacco leaves developed a great many more lesions when the plants were placed in a moist chamber before and after inoculation, than when they were placed in a moist chamber only after inoculation. However, under conditions of our test we have not been able to obtain such results. On February 13, 1940, at 11 a.m., 2 potted plants were placed in a moist chamber, where the leaf surfaces became wet and remained covered with moisture. Twenty-four hours later, a bacterial suspension was poured onto the left side of each leaf, both upper and lower surface. Stomata were open. One plant was then returned to the moist chamber for an additional 24-hour period; the other was left in the greenhouse atmosphere. Only a very few wildfire spots developed, usually at an injured place on the leaf. The right side of all leaves was inoculated at the same time as the left side, but the bacterial suspension was atomized onto the lower surface from a distance of 2 inches. Severe infection developed, showing that the leaves were susceptible.

Two similar plants, which had not been kept moist before inoculation, were inoculated at the same time. One was then placed in the moist chamber for 24 hours, and the other kept in the greenhouse. The same degree of infection developed on these plants as on those kept moist before inoculation. This experiment was repeated 5 times with similar results.

It is thought that bacteria present on wet leaves probably fail to gain entrance because of their inability to pass from water on the leaf surface into air spaces in the stomatal pores.

INVASION OF WATER-SOAKED LEAF TISSUES

Clayton (3) stated that "water-soaked areas facilitated invasion by bacteria but that by far their most important effect was in facilitating the spread of the bacteria through the leaf tissues after infection has occurred." He reported that water-soaking must persist for a period of 24 to 48 hours in order to break down resistance of the leaves. Braun and Johnson (1) later reported that in tobacco seed beds a close relation was found between the amount of water-soaking and the extent of natural infection and invasion with *Bacterium angulatum*. In the present report, water-soaked tissues (water-soaked for only a few minutes) are considered in their role as pathways for the bacteria to enter leaves, rather than as prolonged treatment to break down the resistance of tissue or to spread bacteria through tissue of invaded leaves.

To determine whether surface bacteria can invade leaves water-soaked for only a short time, the following tests were made.

Leaves Water-soaked at Inoculation

On July 28, 1939, at 3 p.m., stomata were open. Two interveinal areas of 4 leaves each of a tobacco plant in the field were water-soaked. A suspension of *Bacterium tabacum* was then poured onto the entire lower surface of each leaf. All the water-soaking disappeared within 15 minutes. By August 4 the areas that had been water-soaked and inoculated were dead, dry, and brown, with a yellow halo surrounding the dead area. It is believed that the extensive necrosis was caused by coalescence of the numerous infections resulting from invasion of bacteria through thousands of stomata. (If a dilute suspension of bacteria was used (1–1000) typical scattered isolated wildfire spots resulted.) There was no infection on the parts of the leaves that had been inoculated without being water-soaked (similar to figure 1). Four other leaves were water-soaked but not inoculated. They showed no injury at any time. This experiment was repeated 9 times with similar results.

Like results were obtained with *Bacterium angulatum*. The infection in the water-soaked tissue was, however, not so extensively necrotic as that produced by *B. tabacum*. Infection with *B. angulatum* usually resulted in numerous crowded angular leaf spots but with little coalescence of the spots.

Further tests showed that the results were essentially the same whether the leaves were water-soaked through the lower or upper surface, and whether the bacterial suspension was poured onto the surface through which water-soaking took place or the opposite surface. Stomata are present on both surfaces, and water-soaking apparently floods the entire thickness of the leaf.

Leaves Water-soaked Before Inoculation

If water-soaked tissue was permitted to lose the water-soaked appearance before bacterial suspension was poured onto the leaf surface, the bacteria

FIG. 1. Invasion of water-soaked areas of a tobacco leaf by *Bacterium tabacum*. The middle three interveinal areas of the right side of the leaf were water-soaked at 10 a.m. September 29, 1939. The water-soaked condition disappeared by 10: 15 a.m. The middle three interveinal areas of the left side were then water-soaked, and a bacterial suspension was poured immediately over the entire lower surface of the leaf. Water-soaking on the left side disappeared within 15 minutes. By October 4 only the areas which were water-soaked at the time of inoculation were dead, brown and dry with a yellow halo. Previous water-soaking had no effect on susceptibility of the leaf to invasion by the bacteria. It is believed that the extensive necrosis was caused by coalescence of infections produced by invasion of thousands of stomata (more than 80,000 per square inch) by the concentrated bacterial suspension. Photographed October 6, 1939.

did not invade the leaf. This is shown by the following test. Three interveinal areas on the left side of a leaf were water-soaked at 2:45 p.m. August 5, 1939. Stomata were open. Water-soaking disappeared by 3 p.m., at which time 2 interveinal areas on the right side of the leaf were water-soaked. Bacterial suspension was immediately poured over the entire lower surface, both left and right sides. Water-soaking on the right side of the leaf disappeared by 3:15 p.m. Infection failed to occur on any part of the leaf except the areas that were water-soaked and inoculated while water-

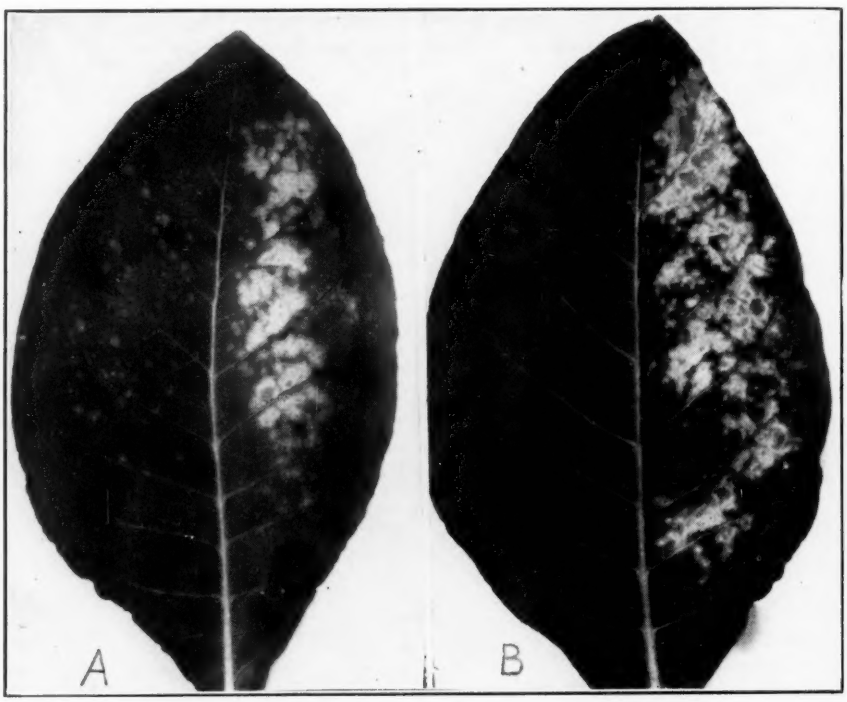

FIG. 2. Bacterial invasion of leaves sprayed with water but not visibly water-soaked. A. Left side atomized with sterile water with atomizer 2 inches from lower surface, and bacterial suspension (*Bacterium tabacum*) immediately poured onto lower surface. B. Bacterial suspension poured onto lower surface of left side without previous spraying with water. Right side of both leaves atomized with bacterial suspension, atomizer 2 inches from lower surface. Inoculated Oct. 9, 1939. Photographed Oct. 17, 1939.

soaking was still visible. By August 9 these were dead, dry, and brown, with a yellow halo. This experiment was repeated 4 times, with the same results (Fig. 1).

INVASION OF LEAVES SPRAYED WITH WATER BUT NOT VISIBLY WATER-SOAKED

The question naturally arises whether there can be enough free water in the stomatal pores and in leaf tissues to permit bacterial entrance in the absence of visible water-soaking. To answer this question the following test

was made. On October 5, 1939, the left side of each of 3 leaves was atomized with sterile water, the atomizer being held 2 inches from the lower surface. There was no visible water-soaking. A suspension of *Bacterium tabacum* was poured onto the lower surface immediately. Within a week there was a considerable number of wildfire spots on these leaves. As controls the left side of each leaf of a second series was inoculated at the same time by pouring bacterial suspension onto the lower surface, without previous spraying with water. No infection developed. The left side of each leaf of a third series was atomized with sterile water, but not inoculated. There was no sign of injury at any time. The right side of each leaf of all 3 series was atomized with bacterial suspension, the atomizer held 2 inches from the lower surface. Severe infection developed on all leaves, showing that all were equally susceptible (Fig. 2). The experiment was repeated 5 times. In 4 cases, the results were similar to these, but in 1 experiment there was total absence of infection on leaves sprayed with water before the bacteria were placed on the surface. The explanation is ventured that, for some reason (probably stomata not open sufficiently), water in sufficient amount was not forced into the stomatal pores and intercellular spaces. The same experiment was repeated 3 times with *Bacterium angulatum*. In each case some infection occurred on leaves sprayed with water (but not visibly water-soaked) before the bacterial suspension was applied.

The results of these tests are interpreted as meaning that when a leaf is sprayed with water, some of the water is driven through the stomata into the substomatal cavities. Not enough water is injected to produce visible flooding or water-soaking, but enough may be injected to form a passage for some of the bacteria subsequently placed on the leaf surface.

DISCUSSION

It is generally believed that in natural field infection *Bacterium tabacum* and *B. angulatum* usually invade tobacco leaves through stomata (2, 4, 7). However, under usual field and greenhouse conditions, bacteria placed on the leaf surface do not produce infection, even though the stomata be open and the leaf surface be kept moist for several hours after inoculation. It appears that the bacteria are unable to pass from water on the leaf surface into the stomatal pores and intercellular spaces. If a continuous passage of liquid exists for a short time from the outer leaf surface, through the stomata, into the intercellular spaces, then bacteria on the leaf surface do gain admission. Temporary natural or artificial water-soaking apparently provides such a liquid passage, for bacteria can infect water-soaked leaves. Natural water-soaking of tobacco leaves is known to occur in plant beds and in the field. In the writers' opinion water-soaked tissues may play an important role as temporary passageways for bacteria to enter leaves, rather than as a prolonged treatment to break down physiological resistance of

plants or to spread bacteria within the tissues, as has been suggested by Clayton (3).

Furthermore, experiments show that bacteria, present on the leaf surface, can sometimes invade leaves that are atomized with sterile water just before inoculation, even though the leaves are not visibly water-soaked. This is interpreted to mean that atomizing with water forces some water into the intercellular spaces without actually water-soaking or flooding the tissues. The water on the walls of guard cells and mesophyll cells adjoining the substomatal chambers probably makes a continuous film with the water on the outer surface through the stomata, and permits bacteria to enter. It is not known whether the bacteria gain admission by swimming or whether they are carried in by some physical force.

Nor is it known whether leaf tissues may, under some natural conditions, contain liquid in the intercellular spaces in amount sufficient to serve as a route for bacterial entrance, but not sufficient to produce visible water-soaking. It is conceivable that, under certain natural conditions, water vapor may condense on the surfaces of guard cells and mesophyll cells or that liquid may be secreted internally without producing visible water-soaking. If stomata are open, such liquid could perhaps serve as a passageway for the bacteria to enter leaves, in the absence of visible water-soaking or flooding of tissues.

The authors' present conception of the conditions necessary for bacterial infection of tobacco leaves is as follows: The stomata must be open (or perhaps at times wounds may serve as openings). The bacteria must be driven into the leaf through the stomata by the force of atomizing or of wind-blown rain, or there must be continuous liquid from the outer leaf surface, through the stomata, into the intercellular spaces, to act as a passageway for the bacteria. The excess internal liquid may be visible (water-soaked or flooded tissues), or it may be invisible, and it need exist for only a few minutes. It must be emphasized that in the field these conditions are not satisfied every time leaves are wet, and, therefore, infection does not occur with every rain, even if pathogenic organisms are on the leaves.

SUMMARY

Infection did not develop in the greenhouse or field when a bacterial suspension (*Bacterium tabacum*) was placed on the surface of leaves, even if the leaves were kept moist some time after inoculation; *i.e.*, bacteria did not swim through stomata to produce infection. However, infection did occur when bacterial suspension of *B. tabacum* or *B. angulatum* was placed on the surface of water-soaked leaf tissues. Sustained water-soaking was not necessary. If the inoculum was applied after water-soaked tissues lost the water-soaked condition, infection did not occur. Furthermore, if leaves were sprayed (but not water-soaked) with sterile water prior to inoculation, bacterial suspension then poured onto the sprayed surface produced consid-

erable infection. The writers interpret these results as meaning that before bacterial infection of tobacco leaves can occur stomata must be open, and a liquid passage must exist between the outer leaf surface and the intercellular spaces; or, if this passageway does not exist, the bacteria must be injected forcefully through the stomata.

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RELATION OF PARTICLE SIZE TO FUNGICIDAL VALUE AND TENACITY OF TWO "INSOLUBLE" COPPER FUNGICIDES[1]

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INTRODUCTION

An improvement in the fungicidal value and tenacity of the "insoluble" copper fungicides is to be desired. The latter materials have been developed partly as substitutes for Bordeaux mixture, but have lacked its fungicidal value and tenacity. Copper carbonate and "Tri-Basic" copper sulphate were the two "insoluble" copper materials in which the writer studied the relation of particle size to fungicidal value and tenacity.

MATERIALS

Four samples of copper carbonate and two of "Tri-Basic" copper sulphate were studied. The samples were received as powders, and varied in their fineness.

Physical data concerning the samples are given in table 1. The colors are according to Ridgway (11).

TABLE 1.—*Physical data of the copper compounds used in this study*

Material	Mean particle diameter	Color	Copper	Specific gravity
	μ		*Per cent*	
Dense copper carbonate, A	5.53	Bluish gray-green	56.5	3.86
Dense copper carbonate, B	5.26	Bluish gray-green	56.5	3.86
Light copper carbonate, C	3.13	Pale sulphate-green	56.0	3.68
Light copper carbonate, D	2.68	Pale sulphate-green	56.0	3.68
"Tri-Basic" copper sulphate—coarse	4.07	Turquoise-green	53.5	3.50
"Tri-Basic" copper sulphate—fine	2.54	Turquoise-green	53.5	3.50

METHODS

Technique for Measuring Particle Size

The mean particle diameter, with certain exceptions, was determined by means of the microprojector method used by Heuberger and Horsfall (5). The number of particles per 100 of 20 μ squares was counted directly on the screen and no attempt was made to prove the Poisson distribution of the particles and to use Thorndike's chart. Magnification was 1467×. This

[1] The writer expresses his thanks to the Tennessee Copper Co., Copperhill, Tenn., for supplying all samples used in this study, to Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station, for invaluable help in connection with the study, and to Dr. James G. Horsfall, of the Connecticut Agricultural Experiment Station, for examining the manuscript.

gave a mean particle size of 2.49 μ for a commercial sample of Yellow Cuprocide (93-per cent cuprous oxide). This is somewhat larger average particle size for this material than Heuberger and Horsfall report (5). However, they used a magnification of 1900 diameters and, by thus including finer particles than were counted in the present study, they would get a smaller mean particle size.

All materials were pasted in a small amount of 50-per cent alcohol-water solution and then diluted with distilled water.

Technique for Measuring Fungicidal Value

The fungicidal value was determined according to the tentative specifications of the current committee of The American Phytopathological Society for the slide-moist-chamber method of testing protective fungicides, and on a standard Bordeaux mixture for laboratory tests and for the determination of the Bordeaux coefficient.

The settling tower (10) was employed to apply the materials to the cellulose-nitrate-coated slides. Five deposits per material per test were used. The test fungus was *Macrosporium sarcinaeforme* Cav. The spore concentration was adjusted to 5000 per cc. by means of a blood-counting cell, except for the tests with "Tri-Basic" copper sulphate, in which it was adjusted to 10,000 per cc. The data were plotted on logarithmic-probability paper, whence the LD50 was obtained.

Technique for Measuring Tenacity

The tenacity of the fungicides and a tenacity coefficient were determined according to the method of Heuberger (3).

EXPERIMENTAL DATA

The present paper is concerned only with laboratory studies on the relation of particle size to fungicidal value and tenacity. The data were analyzed by the analysis-of-variance method (12).

Four Copper Carbonates

Fungicidal Value. The Bordeaux coefficient was determined for each of the 4 samples of copper carbonate as received. Four replicates were made on different days. The particle size and Bordeaux coefficients are listed in table 2. The Bordeaux coefficient is derived from the following formula (8):

$$\text{Bordeaux coefficient} = \frac{\text{LD50 deposition of copper in Bordeaux mixture}}{\text{LD50 deposition of toxicant in fungicide X.}}$$

Thus the closer the Bordeaux coefficient approaches unity the closer fungicide X approaches Bordeaux in fungicidal value.

TABLE 2.—*Effect of particle size on fungicidal value and tenacity of four copper carbonates. (Each figure is the mean of four replicates)*

Material	Particle size[a]	Bordeaux coefficient[b]	Tenacity coefficient[c]
	μ		
Copper carbonate, A	5.53	0.047	0.370
Copper carbonate, B	5.26	0.036	0.403
Copper carbonate, C	3.13	0.254	0.492
Copper carbonate, D	2.68	0.276	0.449
Bordeaux mixture	.....	.....	0.835

[a] The difference in means of A and B is 0.27 μ; of B and C, 2.13; of C and D, 0.45. The least significant difference, at the 5% level, is 0.55 μ.

[b] The difference in means of A and B is 0.011; of B and C, 0.218; of C and D, 0.022. The least significant difference, at the 5% level, is 0.102.

[c] The difference in means of copper carbonates A and B is 0.033; of B and C, 0.089; of C and D, 0.043; of copper carbonate D and Bordeaux mixture, 0.386. The least significant difference, at the 5% level, is 0.094.

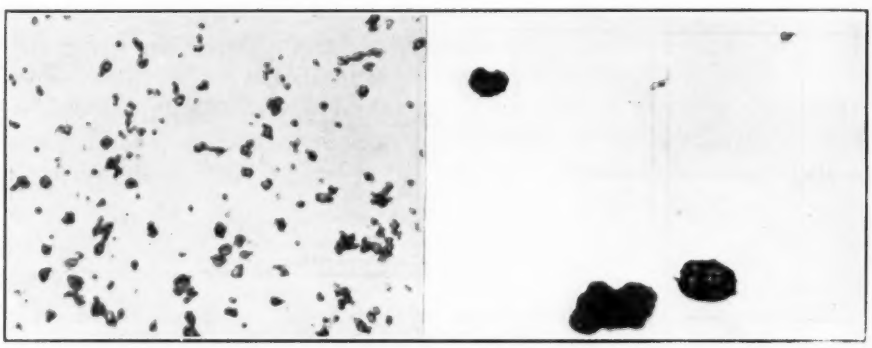

FIG. 1. Photomicrographs of approximately equal weights per unit area of two samples of copper carbonate. Mean particle diameter of samples, left to right, is 2.68 μ; 5.26 μ.

From the data in table 2 we see that an inverse ratio exists between the mean particle diameter and the fungicidal value of the copper carbonates, as measured by the Bordeaux coefficients. That is, as particle size decreases fungicidal value increases. Such relationship has been shown to exist for cuprous oxide (5) and, in the few cases where sound data could be obtained, for a series of "insoluble" copper materials (6).

In this study a significant difference in fungicidal value occurred only when there was a significant difference in the mean particle diameter; that is, between copper carbonates A and B as one group and C and D as another group of smaller particle size. This difference in particle size is vividly illustrated in figure 1. Approximately equal weights of copper carbonate per unit area were obtained for the photomicrographs. This was accomplished by applying equal suspensions of the carbonates to a blood-counting cell and photographing through the cell.

Tenacity. The tenacity of the copper carbonates is seen to increase somewhat as the mean particle size decreases (Table 2). While this difference is not great, hardly being significant, it must be remembered that all samples contained a range of particle sizes, from colloidal on up. The fraction of finer material in samples A and B would tend to minimize the differences in fungicidal value and tenacity between these and samples C and D. This opinion is supported by the data in table 3. Here, the fine fraction of a sample of copper carbonate had greater fungicidal value and tenacity than had the coarse fraction.

Fractionated Copper Carbonate

A sample of copper carbonate was fractionated by elutriation, into definite particle-size classes and their fungicidal value and tenacity determined. This should allow greater precision in the determination of the relation of particle size to fungicidal value and tenacity. The data are presented in table 3.

TABLE 3.—*Effect of particle size on fungicidal value and tenacity of fractionated copper carbonate*

Copper carbonate	Particle size[a] in microns (Mean of 3 replicates)	Bordeaux coefficient[b] (Mean of 4 replicates)	Tenacity coefficient[c] (Mean of 4 replicates)
Coarse fraction	2.06	0.160	0.393
Medium fraction	1.17	0.391	0.512
Fine fraction	0.83	0.534	0.608
Bordeaux mixture	.....	.....	0.769

[a] The difference in the means of the coarse and medium fractions is 0.89; of the medium and fine fractions, 0.34. The least significant difference, at the 1% level, is 0.27.

[b] The difference in the means of the coarse and medium fractions is 0.231; of the medium and fine fractions, 0.143. The least significant difference, at the 5% level, is 0.138.

[c] The difference in the means of the coarse and medium fractions is 0.119; of the medium and fine fractions, 0.096; of the fine fraction and Bordeaux, 0.161. The least significant difference, at the 5% level, is 0.203.

The fractionation of the copper carbonate was not so complete as desired, but the results show that as the particle size of a given material decreases, its fungicidal value increases. Another important consideration is that, under the conditions of these tests, the tenacity of the fungicide increases as the particle size decreases. If these laboratory studies are a reliable indication of field performance, it would be expected that the protective value of copper carbonate would vary inversely with particle size. This suggestion is exactly supported by the data of Twentyman (13) on field performance of copper carbonate. He found that the finer fractions of a standard copper carbonate gave better control of bunt of wheat than did the coarser fractions.

Heuberger and Horsfall (5) found the protective value of cuprous oxide in the field and in the greenhouse to vary inversely with particle size.

"Tri-Basic" Copper Sulphate

The fungicidal value and tenacity of two samples of "Tri-Basic" copper sulphate were determined. The mean particle diameter of the "fine" sample was 2.54 μ; that of the "coarse" sample was 4.07 μ. The data are presented in table 4.

TABLE 4.—*Effect of particle size on fungicidal value and tenacity of "Tri-Basic" copper sulphate. (Each figure is the mean of three replicates)*

Material	Bordeaux coefficient[a]	Tenacity coefficient[b]
"Tri-Basic" copper sulphate, 4.07 μ particle size	0.181	0.301
"Tri-Basic" copper sulphate, 2.54 μ particle size	0.266	0.507
Bordeaux mixture	.....	0.842

[a] The difference in the means of the two samples is 0.085. The least significant difference, at the 5% level, is 0.178.

[b] The difference in the means of the 4.07 μ size and the 2.54 μ size of "Tri-Basic" copper sulphate is 0.206; of the 2.54 μ size and Bordeaux mixture, 0.335. The least significant difference, at the 5% level, is 0.169.

With the "Tri-Basic" copper sulphate, as with the copper carbonates, the fungicidal value and tenacity increased with decreasing particle size. These results show that a further improvement in the insoluble copper fungicides can be achieved by reduction of the particle size.

DISCUSSION

The data presented here show that the fungicidal value and tenacity of copper carbonate and "Tri-Basic" copper sulphate are increased as the mean particle size of the materials is decreased. With copper carbonate this relationship is more clear-cut with the particle size fractions of a given sample than with the several samples of different particle size. This fact emphasizes the desirability of determining the range in particle size of a material as well as its mean particle size in studies of this kind. A narrow range in particle size would be desirable.

The final test of a protective fungicide is its performance in the field. Although in the present study the writer obtained no data on the relation of particle size to protective value, other investigators have done so (5, 13). They found that a decrease in particle size resulted in better protective value. It seems logical that such a relationship would hold true in the present case. One report is to the effect that increasing the fineness of cuprous oxide beyond 325-mesh does not increase its fungicidal value (1). The statement is misleading, however, because 325-mesh measures neither fine-

ness nor coarseness; it merely separates a material into two fractions. One fraction will pass through the screen, the other will not.

SUMMARY

A laboratory study was made of the relationship of particle size to fungicidal value and tenacity of copper carbonate and "Tri-Basic" copper sulphate.

An inverse relationship was found between the particle size and the fungicidal value and tenacity of both insoluble copper materials; that is, fungicidal value and tenacity increased as the size of the particles decreased.

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TEMPERATURE AS IT AFFECTS SPORE GERMINATION IN THE PRESENCE OF COPPER AND SULPHUR

W. D. McClellan

(Accepted for publication July 15, 1941)

The effect of temperature on toxicity of fungicides has been studied to a limited extent only. The general opinion has been that as the temperature increases the toxicity of a fungicide increases and that depression of temperature begets a corresponding reduction in toxicity. The optimum temperature for the growth of the organism involved was not considered. Clark (2), however, stated that the temperature at which a toxic material is applied is very important. In a number of experiments in which the toxicity of mercuric chloride to *Aspergillus* was tested, he concluded as follows: "In general, then, mercuric chloride may be said to be more effective either above or below the optimum temperature for the development of the organism or organisms to be checked; . . . This is probably true of all antiseptics which, like mercuric chloride, check or destroy by precipitation of the contents of the living cell. It *may* be true of all toxic agents." No data were presented to substantiate these statements. Brooks (1), studied the relation of temperature and toxic action of sulphuric acid, nitric acid, and copper sulphate solutions in sugar-beet decoction to spore germination and growth of *Botrytis vulgaris, Monilia fructigena, Sterigmatocystis nigra, Mucor mucedo*, and *Penicillium glaucum*, and arrived at the same conclusion. Doran (3) found some dilutions of copper fungicides were toxic when the temperature was near the minimum or maximum for the germination of the spores he employed and the same dilutions were not toxic at the optimum temperatures for the germination of these spores. He states: "We cannot consider a fungicide toxic unless it is toxic under optimum conditions for spore germination." The data presented in this paper in the form of graphs show that copper sulphate and a particulate sulphur inhibit spore germination least at temperatures which are optima for the germination of spores of the organisms concerned.

METHODS

Conidia of *Sclerotinia fructicola* (Wint.) Rehm, *Alternaria solani* (E. and M.) Jones and Grout, *Venturia inaequalis* (Cke.) Wint., and *Sphaerotheca pannosa* (Wallr.) Lév. var. *rosae* Wor., were used in this study as well as urediospores of *Uromyces caryophyllinus* (Schr.) Wint. Spores of *Sclerotinia fructicola* and of *Alternaria solani* were obtained from cultures that had been growing on potato-dextrose agar for 8 to 12 days. Those of *Venturia inaequalis* were from cultures approximately 2 weeks old produced on cheesecloth wicks by the method of Palmiter (6). Urediospores of *Uromyces caryophyllinus* were obtained from Joan Marie carnation plants, which were naturally infected in the greenhouse. Conidia of *Sphaerotheca*

pannosa var. *rosae* were obtained from actively sporulating lesions on greenhouse roses.

For each organism, except *Sphaerotheca pannosa* var. *rosae*, the spores were germinated in distilled water on glass slides in moist chambers. The spore concentration was adjusted to 50,000 per cc. For each temperature employed, 2 drops of the spore suspension were placed on each of 3 sprayed slides and on each of 3 unsprayed slides. Thus, in each repetition of an experiment, spore germination was observed on 6 slides at each temperature. The 3 unsprayed slides served as a check on germination at each of the temperatures. The percentage germination in all cases was determined by counting 100 spores per slide after 24 hours. Temperatures at 3-degree intervals from 6 to 33° C. were used.

When conidia of *Sphaerotheca pannosa* var. *rosae* were employed, portions of young rose leaflets were used as the test surface, since erratic germination occurs on glass slides (4). The number of spores germinating in each 100 counted was determined after 24 hours by observing the spores *in situ* with the aid of a Leitz Ultrapak microscope. Leaflets were cut along the midrib and one half was sprayed by means of the settling tower and the other half left unsprayed as a check. Conidia were dusted over the sprayed and unsprayed half-leaflets. The temperatures employed were 9, 15, 18, 22 and 28° C. For every repetition of a given experiment 3 unsprayed and 3 sprayed half-leaflets were used at each temperature.

The toxicants employed were a proprietary particulate sulphur[1] and a C.P. grade of copper sulphate ($CuSO_4 \cdot 5H_2O$). For a given experiment the toxicant was applied simultaneously to all of the glass slides (or half-leaflets) to be treated, by means of a settling tower similar to the one described by McCallan and Wilcoxon (5). This assured an equal spray deposit on all of the slides in any one experiment. The spray deposit was regulated so as to allow a spore germination of slightly above 50 per cent on the slides at the optimum temperature for spore germination. When the glass slides were used each experiment was repeated 3 or 4 times; when rose leaflets were used the experiment was repeated 8 times.

EXPERIMENTAL RESULTS

Conidia of *Sclerotinia fructicola*, *Venturia inaequalis*, and *Sphaerotheca pannosa* var. *rosae* were used in studying the effect of temperature on the toxicity of sulphur. The percentage germination of the checks at the various temperatures is shown in figure 1, A. The optimum temperature for the germination of the conidia of the 3 organisms was approximately the same, 21° C. In figure 1, B, the germination ratio obtained by dividing the average percentage germination on the treated surfaces by the average percentage germination on the check surfaces at each respective temperature is represented. Thus, the higher the ratio the less effective the toxic agent in

[1] Walcolized Sulphur obtained from Walco Products, Inc., 512 Greenwich St., New York, N. Y.

inhibiting spore germination, and the lower the ratio, the more effective the toxic agent. The data show that the highest ratios occur at approximately the optimum temperatures for spore germination for the three organisms used.

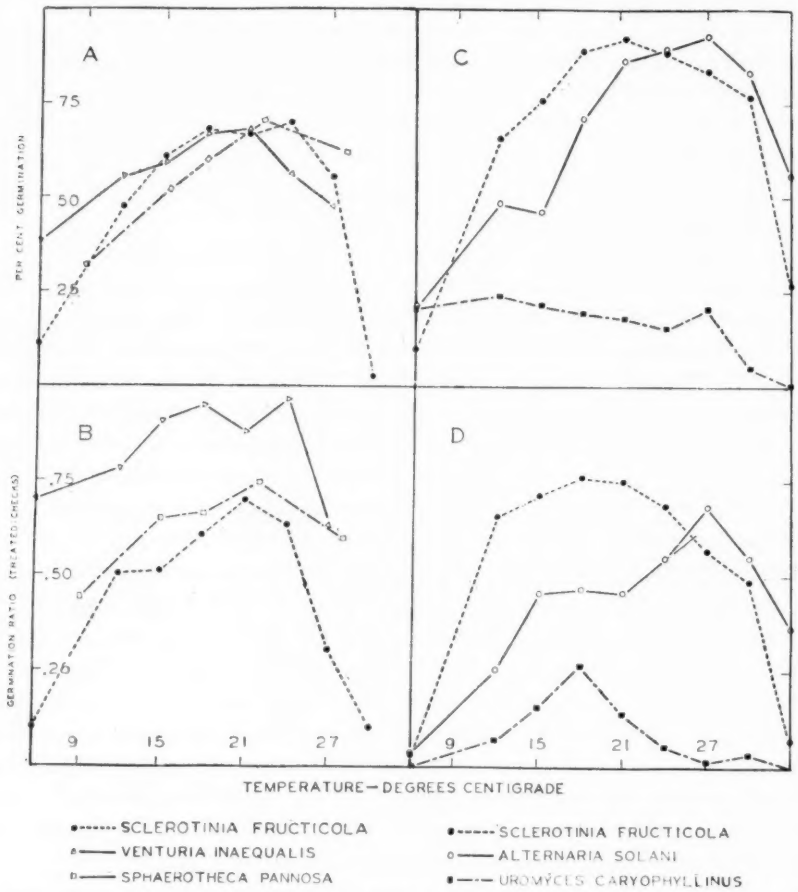

FIG. 1. Graphs showing the percentages of spores germinating on unsprayed glass slides and rose leaflets (A and C) at different temperatures and the ratios of spore germination on sprayed and unsprayed glass and leaf surfaces at the same temperatures (B and D). A. Percentage germination of conidia of *Sclerotinia fructicola* and *Venturia inaequalis* on unsprayed glass slides and of *Sphaerotheca pannosa* var. *rosae* on unsprayed rose leaflets. B. Ratios of the corresponding spore germination on glass slides and on rose leaflets as affected by a particulate sulphur. C. Percentage germination of conidia of *Sclerotinia fructicola, Alternaria solani,* and of urediospores of *Uromyces caryophyllinus* on unsprayed glass slides. D. Ratio of the corresponding spore germination on glass slides as affected by copper sulphate.

In studying the effect of temperature in relation to the effect of copper on spore germination, conidia of *Alternaria solani, Sclerotinia fructicola,* and urediospores of *Uromyces caryophyllinus* were used. The percentage germination of the checks in the respective temperature chambers is graphically illustrated in figure 1, C. It should be noted that the optimum tem-

perature for *Sclerotinia fructicola* is 21° C., while that for *Alternaria solani* is 27. The germination obtained in these experiments with *Uromyces caryophyllinus* was low and cannot be considered conclusive. It merely offers indicative evidence. The ratios are graphically represented in figure 1, D. The maximum ratios (where copper inhibited spore germination least) occur at temperatures of 18, 18–21, and 27° C. for *Uromyces caryophyllinus, Sclerotinia fructicola,* and *Alternaria solani,* respectively.

DISCUSSION AND CONCLUSIONS

The data presented accord with the conclusions of Clark (2) and of Brooks (1), namely, that toxic materials are least effective at the optimum temperature for the organism concerned and that both above and below this optimum temperature these materials are more toxic.

There are many practical implications involved in the question of the relation of temperature to toxicity. Knowledge of this relation may aid the pathologist in recommending spray programs or may help him to explain why a particular fungicide has given excellent results one season but not the next. It may be of considerable importance to the greenhouse man, since, during the winter, he can temporarily adjust the temperature to meet the recommendation. It would seem from these data that in order to destroy a particular pathogen, it would be better to apply a toxic material when the temperature is above or below the optimum temperature for this organism. However, the time required for the killing of an organism by a toxic agent at temperatures below the optimum for that organism would probably be longer than that required at higher temperatures. Therefore, in most cases it would be more desirable to apply a toxic agent when the temperature is above the optimum. An example of a case in which sulphur was more effective at a low temperature than at the optimum was reported by Smith (7). He stated that dusting sulphur was effective in controlling brown rot of peaches under storage conditions at temperatures of 40, 45, and 55° F. (4.5, 7.0, and 13° C.), but that no control was obtained in the lots at temperatures of 65 to 85° F. (18.5 to 29.5° C.). The peaches to which sulphur was not added were readily rotted at all temperatures. If these temperatures are compared with the optimum for *Sclerotinia fructicola* in figure 1, A, or 1, C, it will be seen that the temperatures where no control was obtained were in the range of the optimum for the fungus, whereas those temperatures where control was obtained were much below the optimum.

The data presented in this paper, however, deal only with spore germination. Further studies need to be made of the relationship of temperature and fungicides to the control of plant diseases.

SUMMARY

Studies have been made on the effect of temperature on the fungicidal activity of copper sulphate and a particulate sulphur at temperatures be-

tween 6 and 33° C., inclusive. Copper sulphate was employed against conidia of *Sclerotinia fructicola* and *Alternaria solani* and against urediospores of *Uromyces caryophyllinus*. Sulphur was employed against conidia of *Sclerotinia fructicola*, *Venturia inaequalis*, and *Sphaerotheca pannosa* var. *rosae*.

Copper sulphate and sulphur were least effective at the optimum spore germination temperatures of the organisms employed.

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SOME FACTORS AFFECTING THE INFECTION OF TOMATO SEEDLINGS BY ALTERNARIA SOLANI[1]

W. D. Moore

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INTRODUCTION

During recent years the southern production of vegetable seedlings for transplanting in the North, has developed into an important agricultural industry. Of the several crops grown, the tomato is of greatest importance both in value and in acreage planted. With these field-grown plants, the leaf spot and stem canker caused by *Alternaria solani* (E. and M.) J. and G. has been one of the most important of tomato disease problems. Tomato seedlings grown in the South during the early spring are produced under conditions different from those encountered by plants grown under protection in the North, since they are subject to variable weather conditions and undergo considerable handling during harvesting and packing for shipment. As these factors may influence infection by *A. solani*, the limits of temperature and humidity within which infection may occur and the relation of mechanical injury to infection are of considerable importance in the study of the disease on field grown seedlings.

TEMPERATURE AND HUMIDITY

The influence of temperature and humidity on infection by *Alternaria solani* has been shown by Heald (1) and Rands (3, 4) for potatoes, and by Rands (4) and Weber (5), for tomatoes, in reports dealing with the maturing plant. Kreutzer and Durrell (2) showed that this organism can produce collar rot on tomato seedlings at temperatures greater than the optimum for good plant growth but they did not study the leaf-spot phase of the disease. Both Rands (4) and Weber (5) report that periods of high humidity are followed by the appearance of abundant infection and that fairly high temperatures also are most conducive to the development of the disease.

In the course of investigations of the leaf spot and stem canker caused by *Alternaria solani* on tomato seedlings in southern plant fields, data have been recorded on the effect of different periods of high humidity on the development of leaf spot in order to ascertain the duration necessary for an appreciable amount of infection. Studies also have been made with regard to the lower range of temperatures at which leaf-spot infection may occur.

In order to determine the influence of varying periods of high humidity

[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Georgia Coastal Plain Experiment Station, Georgia Agricultural Experiment Station, Georgia Department of Entomology, New Jersey Agricultural Experiment Station, and Indiana Agricultural Experiment Station.

on leaf-spot infection, several tests were conducted in the greenhouse in which 4 sets of inoculated tomato plants were held at a fairly uniform temperature. Three of these sets were incubated for 4, 8, or 16 hours' time in moist chambers having an atmosphere of 98–100 per cent relative humidity. During the time that the plants were not in the high-humidity chambers, they were held in a greenhouse atmosphere that varied in relative humidity from 40 to 80 per cent. In the temperature studies, the humidity was held approximately constant and the temperature was maintained at different mean levels. Plants of equal age and vigor were used in all experiments. Inoculations were made at the beginning of each test by spraying a heavy spore suspension of *Alternaria solani* over the foliage with an atomizer. Leaf-spot counts were made after an incubation period of 4 to 10 days.

The influence of prolonged high humidity development of leaf spot is shown in table 1. From these data it was found that periods of high relative

TABLE 1.—*The development of A. solani leaf spot on inoculated tomato plants following incubation in an atmosphere of 98–100 per cent relative humidity for numbers of hours shown. (Eight 3-plant cultures per treatment in each experiment)*

Test numbers	Date inoculated	Date counted	Mean number of leaf spots per culture			
			0 Hours	4 Hours	8 Hours	16 Hours
1	2/14/41	2/20/41	0	5.3 ± 0.8[a]	47.8 ± 18.8	285.2 ± 55.5
2	3/10/41	3/24/41	0	3.6 ± 1.0	24.6 ± 5.0	166.7 ± 18.9
3	3/24/41	4/ 4/41	0	6.8 ± 1.6	21.3 ± 2.9	154.3 ± 20.2

[a] Standard error of the mean.

humidity for as little as 4 hours were sufficient to induce appreciable leaf-spot incidence, but no infection occurred when inoculated plants were held at the lower humidities of the open greenhouse. Disease development increased, though not proportionately, as the hours of high relative humidity were increased. Daily observations of all lots of plants in these experiments showed that humidity sufficient to cause an accumulation of dew on the tomato foliage is necessary for infection by *Alternaria solani.*

TABLE 2.—*Development of A. solani leaf spot on inoculated tomato plants incubated at a constant humidity level but at different temperatures. (Eight 3-plant cultures per treatment in each experiment)*

Test numbers	Date inoculated	Date counted	Mean number leaf spots per culture	
			Mean temperature range 54–62° F.	Mean temperature range 74–82° F.
1	1/23/41	1/31/41	142.6 ± 13.0[a]	446.8 ± 95.6
2	2/18/41	2/26/41	225.3 ± 28.3	278.7 ± 37.8
3	3/18/41	3/26/41	62.6 ± 15.3	190.0 ± 27.0
4	3/28/41	4/ 7/41	95.0 ± 30.7	153.2 ± 24.1

[a] Standard error of the mean.

In tests where approximately uniform humidity was maintained at a level sufficiently high to induce infection by *Alternaria solani*, and the temperature was held at mean levels between 54° and 62° and 74° and 82° F., leaf-spot infection increased as the mean temperature was increased, with a rather abrupt increase at 60° F. or above. From the data shown in table 2, it is apparent that leaf-spot infection in the field, under favorable conditions of humidity, will develop more abundantly as the mean daily temperatures rise during the advancing spring season. It is of particular interest, however, to note that appreciable infection by *A. solani* occurred at the lowest temperatures included in these tests, some of which were materially lower than the daily mean ordinarily experienced in the plant fields of the South. From the standpoint of the commercial grower, this is of considerable importance, since it suggests that infection may take place under all field growing temperatures, provided sufficient moisture is present on the leaves.

FOLIAGE INJURY

In the normal process of harvesting, packing, and shipping tomato seedlings, some injury to the foliage and stems is unavoidable. In view of the fact that both humidity and temperature are quite favorable for infection in the shipping crates, several tests were made to determine what influence foliage injury might have upon increased disease incidence. In these tests, one series of plants suffered no injury, the foliage of another was subjected to a unit (300 g.) quantity of sand per series from a small sand-blowing machine, and that of still another to two units of sand. In no case was there visual evidence of appreciable injury to the epidermis of the leaves after the sand blowing was completed. Following this treatment, all lots were inoculated with approximately equal amounts of a heavy spore suspension of *Alternaria solani* and held for periods of from 5 to 8 days in a moist chamber, where both humidity and temperature favored infection. Leaf-spot infection (Table 3), under the conditions of these tests, was increased by mechanical injury immediately before inoculation. In view

TABLE 3.—*The development of A. solani leaf spot on tomato plants inoculated following different degrees of mechanical injury. (Eight 3-plant cultures per treatment in each experiment)*

Test numbers	Date inoculated	Date counted	Leaf spots per culture after treatment		
			Control	Sand abrasion	
				300 g. sand	600 g. sand
1	1/21/41	1/28/41	107.5 ± 31.5[a]	619.3 ± 76.0	879.7 ± 97.0
2	1/21/41	1/29/41	339.2 ± 39.3	594.8 ± 72.1	743.8 ± 101.5
3	3/3/41	3/10/41	80.8 ± 17.7	96.3 ± 20.4	129.6 ± 17.2

[a] Standard error of the mean.

of the plant injury caused during certain periods of blowing sands, also the unavoidable injury incident to the normal process of harvesting and

packing tomato seedlings, it appears that leaf spot may be enhanced at certains times by these factors.

STEM INJURY

From the standpoint of the northern tomato grower, the stem canker phase of *Alternaria solani* infection on tomato seedlings is a matter of much greater importance, than is leaf spot. Since seedling stems, as well as foliage, are invariably subjected to slight mechanical injuries during harvesting and shipping, a series of inoculation tests was made to determine the effect of this injury on subsequent development of stem canker. As in the case of the foliage experiments, certain series were inoculated without injury to the plant stems, others were inoculated after the stems were subjected to a unit (432 g.) quantity of sand per series from a sand-blowing machine, and still others after being subjected to 2 units of sand. All lots were held under the same conditions of temperature and humidity for periods of 3 to 5 days, and then read for stem canker (table 4). From these data, it appears

TABLE 4.—*The development of A. solani cankers on tomato plants subjected to different degrees of injury prior to inoculation. (Eight 3-plant cultures per treatment in each experiment)*

Test numbers	Date inoculated	Date counted	Stem cankers per culture after treatment shown		
			Control	Sand abrasion	
				432 g. sand	864 g. sand
1	11/18/40	11/22/40	0	4.8 ± 1.0	16.8 ± 3.2
2	11/25/40	11/29/40	1.6 ± 0.5[a]	83.3 ± 8.7	111.2 ± 13.2
3	1/11/41	1/14/41	0	14.0 ± 7.5	40.8 ± 8.4
4	1/13/41	1/17/41	0	52.0 ± 5.5	88.2 ± 8.2

[a] Standard error of the mean.

that stem canker development, under conditions of optimum temperature and humidity, is likely to increase because of injury sustained by the plants during harvesting and shipping.

DISCUSSION

Most of the tomato seedlings produced in the South for northern shipment are grown during March, April, and May. For most plant areas in the southeast, this period is fairly free from excessive rain and high humidity. In the light of data collected on disease incidence under different humidity conditions, it appears that *Alternaria solani* leaf spot may be expected to become seriously important only during periods of prolonged high humidity. Field observations throughout all sections of the plant-producing area of southern Georgia during the period 1937–1940, inclusive, have indicated the validity of this conclusion (Table 5). Since the mean daily temperatures during the spring months rise at a fairly uniform rate each year, disease incidence may be expected to increase with the advance

TABLE 5.—*The number of days and the mean number of hours per day with 4 or more hours of 90–100 per cent relative humidity at Tifton, Ga., during the March 20 to May 20 period for the years 1937 to 1940 together with observations of general field infection of Alternaria solani*

Year	March		April		May		General field infection by *A. solani*
	Number days	Hourly mean per day	Number days	Hourly mean per day	Number days	Hourly mean per day	
1937	7	6.5	18	8.0	10	5.0	Light
1938	8	9.5	17	7.8	6	7.6	Light
1939	11	11.6	25	10.8	19	10.2	Severe
1940	3[a]	9.3	14	6.7	5	5.2	Light

[a] Record for 5 days.

in temperatures during those seasons when humidity conditions are favorable for infection.

During the process of pulling and packing tomato seedlings, some mechanical injury is unavoidable. Since the plants are packed in wet peat moss, a high humidity is created in the shipping crates, particularly near the center, and this is conducive to disease development. In many instances, the crates are subjected to temperatures that are likewise conducive to infections of both the leaves and stems. In the light of the data on the influence of mechanical injury, it appears that, under the above-mentioned shipping conditions, leaf spot and stem canker on tomato seedlings may be increased through mechanical damage to either foliage or stems.

SUMMARY

The principal limiting factor in leaf-spot infection of tomato seedlings by *Alternaria solani*, is humidity. The critical phase of this humidity appears to be the number of hours per day in which the atmosphere is near saturation rather than the daily mean relative humidity for any given period.

Given humidity conditions suitable for *Alternaria solani* infection, leaf-spot incidence increases with the advance in mean temperature within the range studied (to 82° F.). Appreciable infection develops, however, at mean temperatures below those considered suitable for good tomato seedling growth.

Leaf spot and stem canker increases with the degree of mechanical injury to which the plants are subjected prior to inoculation.

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PHYTOPHTHORA CROWN ROT OF LOQUAT

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The loquat, *Eriobotrya japonica* Lindl., has long been grown in California both as an ornamental and as a fruit tree. According to a 1936 survey (2) there were 147 acres of commercial plantings in the State at that time. It is present also in many park, garden, and street tree plantings. In the city of San Jose, California, a survey showed 231 loquat trees had been planted in roadside parking areas. Despite this widespread planting, the crown rot disease due to natural infection by *Phytophthora cactorum* (L. and C.) Schröt. has not previously been reported or described in California. No record of its occurrence elsewhere in the United States has been found.

HISTORY OF CROWN ROT

Dufrenoy (4), in 1927, isolated a *Phytophthora* from loquat crown-rot lesions in Corsica, which he named *P. omnivora parasitica* forma *eriobotryae*. The following year Nicolas and Aggery (6) reported this fungus as a new parasite of the loquat. Tucker (9) suggested that the name of the organism be changed to *P. parasitica*.

Tucker (10) recorded an isolation of *Phytophthora cactorum* from the loquat in Japan in 1932 by T. Tasugi. In 1937, C. O. Smith (8) reported successful artificial inoculations of the loquat and 41 other species of plants with a pure culture of *P. cactorum*, isolated from *Juglans regia*. Baines (1) successfully inoculated flax and peony plants and two varieties of apple with the culture of *P. cactorum* isolated from loquat by Tasugi.

In July, 1939, two 11-year-old loquat trees in the Subtropical Horticulture orchard at the University of California at Los Angeles were found affected with a crown or collar rot. Isolations from the margins of these crown lesions yielded cultures of *P. cactorum*.[1] One of these trees was the Early Red and the other the Advance variety, both budded on seedling loquat stock.

SYMPTOMS OF THE DISEASE

The Early Red tree had two irregular necrotic bark lesions located on opposite sides of the trunk at the crown or collar. A light scraping of the bark revealed the margins of these affected areas. The outer bark tissues of the small but rapidly advancing areas were discolored several inches beyond the zone in which the entire bark was affected to the cambium. These recently invaded tissues were light brown and appeared watersoaked, without any exudation. Brown streaks in the cambium region extended several inches beyond the margin of the affected areas as in the Phytophthora trunk canker or collar rot of apple trees described by Baines (1). The discolora-

[1] The assistance of Dr. C. M. Tucker in the identification of the culture is gratefully acknowledged.

tion of the invaded sapwood and the strong odor of fermentation in the diseased bark, which he mentions, were notable characteristics of this loquat disease. The color of the invaded bark varied from Ridgway's (7) fuscous brown to fuscous black at the surface and from Prout's brown to mummy brown beneath, in contrast with the parrot or cress green color of the surface and the white color of the inner tissues of the healthy bark.

The presence of the disease was first detected by the appearance of the secondary symptoms on the Advance tree (Fig. 1). The initial crown-rot areas had merged, completely girdling the trunk at the soil level and had extended upward to the main branches. Defoliation had started with the loss of the older leaves and progressed until only a tuft of small, yellow, half-grown leaves remained at the ends of the terminal branches. The yellowing and dropping of the leaves accelerated the decline of the tree. The development of flower clusters was checked and the fruit set greatly reduced. This tree died and was removed in January, 1940, before any of the fruit reached maturity.

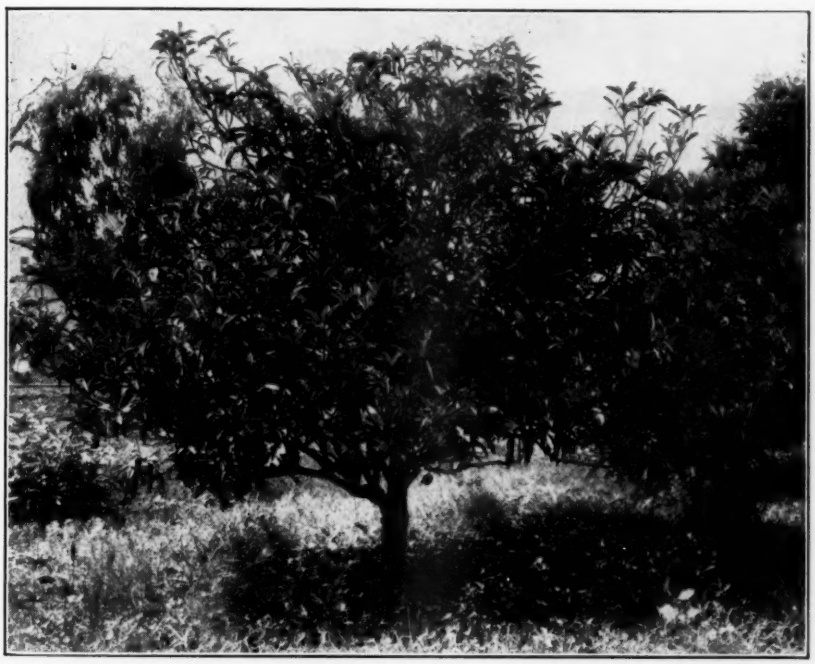

FIG. 1. Advance loquat tree on seedling root girdled by Phytophthora crown and trunk lesions (see Fig. 2). Progressive defoliation has left only clusters of yellowish green, immature leaves at the ends of the branches.

The margins of old inactive cankers on this tree were clearly defined by a bark crack that followed the outline of the lesions. Drying and shrinkage of the dead bark within these areas produced continuous transverse cracks that in some cases extended the full width of the canker. Short, discontinuous, longitudinal cracks broke the surface into small irregular patches of

dry, dead bark. On some parts of the trunk these bark scales curled away from the trunk, loosened, and sloughed off, exposing the wood (Fig. 2).

ISOLATION OF THE CAUSAL FUNGUS

Successful isolations of *Phytophthora cactorum* from infected tissues were made by all of the following methods. Direct transfer of small bits of diseased tissue from active canker margins that had been surface-sterilized with 95 per cent alcohol yielded a number of pure cultures. Blocks of tissue from the advancing margins of cankers were dipped in 95 per cent alcohol, passed through a gas flame, and placed in sterile moist chambers. Transfers of the mycelia from these blocks yielded pure cultures. Pure cultures also were obtained from apples inoculated with diseased loquat bark tissue. A fourth method was used to reisolate the fungus from artificially inoculated stems of small seedling loquat trees. Sections of the diseased stems were supported on a wire screen placed on the top of a moisture dish or bell jar filled with water. Dripping water from a faucet splashed over the partly submerged stem sections. Bits of mycelium, which emerged from the diseased tissue, were transferred to agar in culture tubes. Potato-dextrose agar was used for all cultures.

FIG. 2. Advance loquat, 11 years old showing definite margins, bark cracks and bark shedding of old canker that extended around the trunk.

ARTIFICIAL INOCULATIONS

Pure cultures obtained by these various methods were used to inoculate

fruits of watermelon, quince, apple, and lemon and seedlings of sweet orange, rough lemon, loquat, pineapple guava (*Feijoa sellowiana*) and natal plum (*Carissa grandiflora*). The fruit inoculations were made by inserting mycelium in rind punctures, each fruit having previously been washed with 95 per cent alcohol and placed in sterile moist chambers. The inoculated fruits were held at room temperature (approximately 70° F.). The method described by Smith (8) was used in the inoculation of the 3 seedlings of each kind. A 3/16-inch cork borer was used to make the inoculation wounds. One check plant was used in each group.

Two weeks after inoculation, the infected areas on watermelon fruits averaged 7 cm. in diameter and showed surface growth of mycelium. Pieces of infected tissue, transferred to sterile tap water in Petri dishes, were held 48 hours at room temperature. Microscopic examination of macerated bits of this tissue showed by that time an abundance of typical antheridia, oogonia, oospores, and sporangia of *Phytophthora cactorum*.

Six days after inoculation, 3 quince fruits had developed infected areas

FIG. 3. Typical lesion produced by artificial inoculation of a locust seedling with *Phytophthora cactorum* isolated from naturally infected Early Red loquat tree.

averaging 4 cm. in diameter. Four inoculated apple fruits of the Winesap, Jonathan, Newtown Pippin, Rome Beauty, Bellflower, and Red Delicious varieties, after 9 days, had infected areas averaging from 5 to 7 cm. in diameter. Microscopic examination of rotted apple and quince tissues, after 2 days in sterile water, showed numerous oogonia and oospores but very few sporangia.

The lemon-fruit inoculations, though repeated several times, were negative, as were those of the seedlings of sweet orange, rough lemon, pineapple guava, and natal plum. Inoculation of 6 loquat seedlings on November 21, 1939, resulted in typical lesions varying in length from 2 to 17 cm. The 17 cm. lesion was measured 45 days after date of inoculation; the others,

after 56 days had elapsed. The wounds on the 3 check seedlings had completely healed. One of these typical cankers resulting from an artificial inoculation is shown in figure 3.

DISCUSSION OF ARTIFICIAL INOCULATIONS

Smith (8) reported successful inoculations of lemon fruits and lemon, sweet orange, and loquat trees with the culture of *Phytophthora cactorum* isolated from walnut crown-rot lesions. The negative results of the writer's inoculations with the isolate from loquat suggest that it may be a different physiologic race of *P. cactorum* from that used by other workers. Baines (1) has presented evidence of the existence of physiologic races of this fungus. However, since he also found young Grimes Golden apple trees, 2 to 4 years old, highly resistant to both artificial and natural infection, the negative results from inoculations of the citrus, feijoa and carissa seedlings in this case may not have great significance. It will be necessary to repeat the inoculations using mature trees or plants to obtain further evidence of racial difference.

CONTROL METHODS

Decortication followed by a fungicidal application is the treatment generally recommended (3, 5) and widely used for the control of phytophthora gummosis of citrus trees and crown rot of walnuts. In July, 1939, the crown-rot lesions of the Early Red loquat tree were treated by this method. The bark was scraped lightly to determine the full extent of the infected areas. The dead bark within the areas and the healthy bark 1 to 1½ inches beyond the margins and through the cambium to the wood were removed with a pruning knife. A fungicidal wash of ½ oz. of dry Bordeaux mixture, dissolved in a pint of water, was applied to the treated areas with a brush. In the 2-year period since these areas were treated, neither of them has shown further development.

SUMMARY

A crown rot of the loquat tree, attributable to natural infection by *Phytophthora cactorum*, is described and reported for the first time in this country. Another species of *Phytophthora* had previously been isolated from this host in Corsica and reported as a new parasite of the loquat. *P. cactorum*, isolated from this host in Japan and from walnut crown-rot lesions in California, have been reported able to infect numerous other artificially inoculated host plants.

Irregular brown to black necrotic bark lesions develop at the crown or collar of the tree and extend upward on the trunk and main branches. The bark of old cankers dries, cracks, curls away from the trunk, and sloughs off in patches. In advanced stages of the disease the secondary symptoms appear as a progressive yellowing and dropping of the leaves and general decline of the tree.

Isolation of *Phytophthora cactorum* from the infected tissues was accomplished by 4 different methods. Artificial inoculations of watermelon, apple, and quince fruits and loquat seedlings resulted in infections. Typical cankers developed on the inoculated loquat seedlings.

Decortication, followed by a fungicidal wash, gave effective control of the disease in its early stage.

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THE SUCCESSFUL TRANSMISSION OF PSOROSIS OF CITRUS TREES IN FLORIDA BY BARK GRAFTING

ARTHUR S. RHOADS[1]

(Accepted for publication July 22, 1941)

The convincing demonstration in 1933 and 1934 by Fawcett (2, 3, and subsequent papers) that psorosis of citrus trees is a virus disease engendered an entirely new concept of this peculiar disease that has baffled investigators for many years. The writer has devoted more than a decade to the study of this complex disease in Florida with a view to determining its nature and cause and the possibility of controlling it by the bark-scraping treatment. He has never been able, however, to isolate an organism other than certain ubiquitous fungi commonly associated with it as secondary organisms. In recent years Florida growers have spent considerable time and money in endeavoring to treat psorosis by the bark-scraping method and in making injections of various chemicals into the trunks and limbs of diseased trees, but without success. The writer (6, 7) conducted for 11 years extensive experiments on many trees to determine the effectiveness of bark-scraping in the control of this disease. At the end of this period it could only be concluded that this treatment was very effective in most cases in apparently curing psorosis, or at least temporarily arresting its progress, on orange and grapefruit trees, provided the work be carefully and thoroughly done in the early stage of development of the disease. Even then, however, the disease often developed again in the renewed bark over old treated lesions or at new points on the treated trees in subsequent years. It was found (5) that by the time the disease had reached an intermediate stage of development it almost invariably became systemic and, following the death of areas of the bark, the interior wood of the limbs and trunks of attacked trees usually became extensively invaded and discolored by various secondary fungi that greatly expedited the decline of the trees. These results appear to agree generally with those secured by Fawcett (4) in California and the experience of Doidge and Turner (1) with this disease in South Africa. It is now agreed by all who have given psorosis serious study that it is an infectious disease transmissible through budwood taken from trees that may not show even the bark symptoms of this disease but may be carrying it in a latent form. Usually, from 8–10 years are required for psorosis to manifest itself by the development of the well-known bark symptoms. As Fawcett (2, 3, 4) has shown, however, the leaf symptoms may appear much earlier. Doidge and Turner (1) state that psorosis may continue to develop on individual trees that have been in the orchard for 20 to 30 years, or even longer, without having previously shown bark symptoms. They also state that even psorosis-affected trees that were cut off an inch or two above the

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bud union and had grown into apparently healthy trees, again developed this disease some 7 years later.

In 1935, following the presentation by Fawcett (2, 3) of evidence that psorosis is a virus disease, the writer, lacking opportunity to conduct extensive experiments along this line, made a small series of bark grafts in a few large bearing citrus trees. In one of his periodic treatments of trees for the control of psorosis in a grove at Bonaventure, an unusually fine bark lesion 18 inches long was found on an orange limb 4 inches in diameter. The section of the limb bearing this active psorosis lesion was divided into two pieces, one of which was used on May 16 to make a dozen patch-bark inoculations into the upper central limbs of 5 orange trees about 28 years old in a grove near Courtenay on Merritt Island. Pieces of the diseased bark $\frac{3}{4}$-inch square were removed and inserted on the limbs at points where areas of bark of exactly the same size were removed. The pieces of bark thus used as inoculum were held firmly in place with string and painted with melted paraffin. Three inoculations were made on the limbs of each of 3 trees, 2 on limbs of another, and 1 on a limb of a fifth tree.

The other half of this section of limb bearing the psorosis lesion was used for making a series of 20 cultures, of which 17 remained sterile, 1 developed *Colletotrichum glocosporioides*, 1 *Phomopsis citri* and 1 a slow-growing *Diplodia*-like fungus.

The series of patch-bark inoculations was inspected on October 15, at which time it was apparent that but 6 were successful. These inoculations were inspected at intervals of 2 or 3 times a year, but there was no evidence of the transmission of the bark symptoms of psorosis as late as the end of August, 1937. However, at the next inspection on June 7, 1938, 3 years and 1 month from the date of the inoculations, it was clearly apparent that definite cases of psorosis were developing from some of the successful patch grafts on 3 of the 5 trees. At this time 2 of the 3 inoculations on tree No. 1 showed the typical beginnings of psorosis, the single inoculation on tree No. 2 showed a single small scale of bark at the upper left corner, and of the 3 inoculations on tree No. 4, 2 showed early stage bark scaling typical of psorosis, and the other had developed merely a slight bark scale at the left side.

At the next inspection on May 18, 1939, evidence of the successful transmission of psorosis was much more pronounced. On tree No. 1 the 2 successful inoculations had developed typical psorosis, but the tree was manifesting that chronic wilt and decline designated as blight, and was removed during the summer. The single successful inoculation on tree No. 2 showed a single large scale of bark partly exfoliated at the upper left corner. On tree No. 4 all 3 inoculations showed lesions about 3 inches long and extending $\frac{2}{3}$ around the limbs.

In the inspection of June 10, 1940, the single successful inoculation on tree No. 2 showed more bark scaling, and typical psorosis had developed at the left side of the inoculation. On tree No. 4 a characteristic psorosis

lesion extended all the way around one limb, $\frac{2}{3}$ the way around another, and a slight lesion had developed on the other inoculated limb.

At the inspection on August 27, 1940, just prior to the closing of the Citrus Field Laboratory at Cocoa, the single successful inoculation on tree No. 2 showed a typical psorosis lesion 2 in. long and $1\frac{1}{2}$ in. wide at the left side of the inoculation. On tree No. 4 the lesion on the $2\frac{1}{2}$-in. limb on the west side had attained a length of 5 in. and just about completely encircled it, with very pronounced bark scaling and another separate area of bark scaling developed slightly above. On the inoculated $2\frac{3}{4}$-in. limb on the northwest side a typical psorosis lesion 3 in. long and extending part way around it, mostly on the left side of the inoculation, had developed. The third limb, which was 2 in. in diameter, had developed a typical psorosis lesion 6 in. long and completely encircled it. This was removed and photographed (Fig. 1).

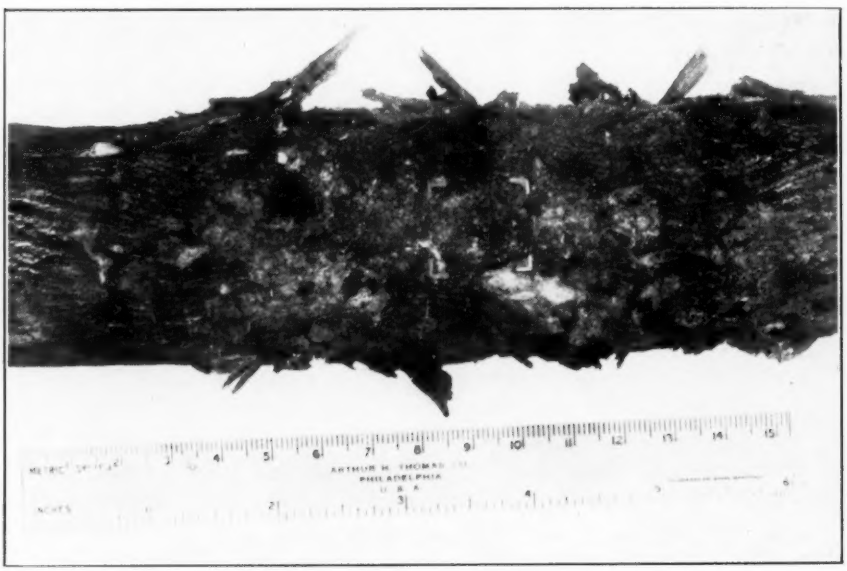

FIG. 1. Typical bark-scaling lesion of psorosis 6 inches long and completely encircling 2-inch orange limb; this developed 5 years after grafting $\frac{3}{4}$-inch square patch of bark (indicated in center) from lesion on another orange tree.

At the final inspection on June 10, 1941, the psorosis lesion on tree No. 2 had attained a maximum length of 3 in. and extended half way around the $2\frac{1}{2}$-in. limb on the left side of the inoculation. On tree No. 4 the lesion on the $2\frac{1}{2}$-in. limb on the west side had completely encircled it and attained a maximum length of 6 in. on one side, with a separate small scaling area above. The lesion on the $2\frac{3}{4}$-in. limb on the northwest side extended half way around it, mostly on the left side of the inoculation, and had a maximum length of 4 in.

It is apparent from the foregoing that psorosis has been definitely trans-

mitted by bark grafting in 6 out of 12 inoculations and the typical bark symptoms of the disease reproduced, their development first being apparent after the lapse of approximately 3 years. Even after the lapse of 3 additional years, however, this slow-developing disease had made very little progress. No bark symptoms of psorosis developed at any points on the 5 trees used in the experiment other than where inoculations were made, and no evidence of psorosis developed in any of the 6 inoculations where the patch of bark used as the inoculum failed to unite with the tissues of the tree. This inoculation experiment furnishes definite proof that psorosis is an infectious disease that may be transmitted from diseased to healthy trees by union of tissues and confirms the work of Fawcett, who has demonstrated that the causal agent is a virus.

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PHLOX RESISTANT TO POWDERY MILDEW[1]

E. B. MAINS

(Accepted for publication July 25, 1941)

The powdery mildew of phlox is placed by Salmon[2] in the species *Erysiphe cichoracearum* DC. It is rather widespread on garden varieties of both the perennial (*Phlox paniculata* L. and *P. maculata* L.) and the annual phlox (*P. drummondii* Hook.). In the vicinity of Ann Arbor, Michigan, it ordinarily becomes noticeable early in the summer and is usually severe by late summer. It may cause considerable defoliation. When this occurs for several years the plants are much weakened. Even when the disease is less severe, the mildew makes plants unsightly, a very undesirable condition for an ornamental.

PERENNIAL PHLOX

In 1933, mildew was unusually severe in the writer's garden. Between several named varieties of perennial phlox there were a number of seedlings. It was noted that one of the latter remained free from mildew throughout the season, while the other plants were severely infected. In the spring of 1934, a division of the resistant plant was transplanted to the greenhouse of the Botanical Garden of the University of Michigan. Here it was studied in comparison with 22 named varieties.[3] In addition 25 seedlings occurring in the vicinity of the resistant plant, and apparently from seed produced by it, also were tested. These plants were repeatedly inoculated with mildew from May through August. Very pronounced differences occurred. All the named varieties were more or less susceptible. The varieties Africa, Commander, Ethel Pritchard, Graf Zeppelin, Lillian, Maréchal French, Miss Lingard, Mrs. W. Van Bueningen, Nicholas Flammel, Paladin (Fig. 1, D). Rijnstroom, R. P. Struthers, Thor, Von Lassburg and Widar were very susceptible. By the first of August, most of their leaves had been killed. The varieties Columbia (Fig. 1, C) Enchantress, Emain Macha, Leo Schlageter, Pastel, Saladin, and Milly Van Hoboken were less susceptible, mildewing somewhat more slowly. By the end of the season their leaves were covered with the fungus. The original resistant selection (H 1) was highly resistant (Fig. 1, A) throughout the entire period. No mildew was evident and the leaves were in good condition at the end of the season. Of the seedlings assumed to have been derived from the resistant plants, 4 were very susceptible, and 4 moderately susceptible. A limited amount of mil-

[1] Paper from the Department of Botany, Botanical Garden and Herbarium of the University of Michigan.

[2] Salmon, Ernest S. A monograph of the Erysiphaceae. Mem. Torr. Bot. Club. **9**: 1–292. 1900.

[3] The results were briefly reported at the 27th annual meeting of The American Phytopathological Society at St. Louis, December 31, 1935. Phytopath. **26**: 101. 1936.

dew developed on 7 plants. Nine seedlings were highly resistant (Fig. 1, B), showing little or no mildew throughout the study.

In 1935, the varieties Columbia, Emain Macha, Miss Lingard, Nicholas Flammel, and Paladin and the most promising resistant selections from the previous studies were planted outdoors in the Botanical Garden where most of them have been under observation since. In 1935, powdery mildew was severe. The reactions of the varieties and selections agreed very closely with those obtained in the greenhouse in 1934. In 1936, the writer was in British Honduras during the summer, and notes were not obtained. In 1937, mildew did not appear until late, and developed slowly. The most

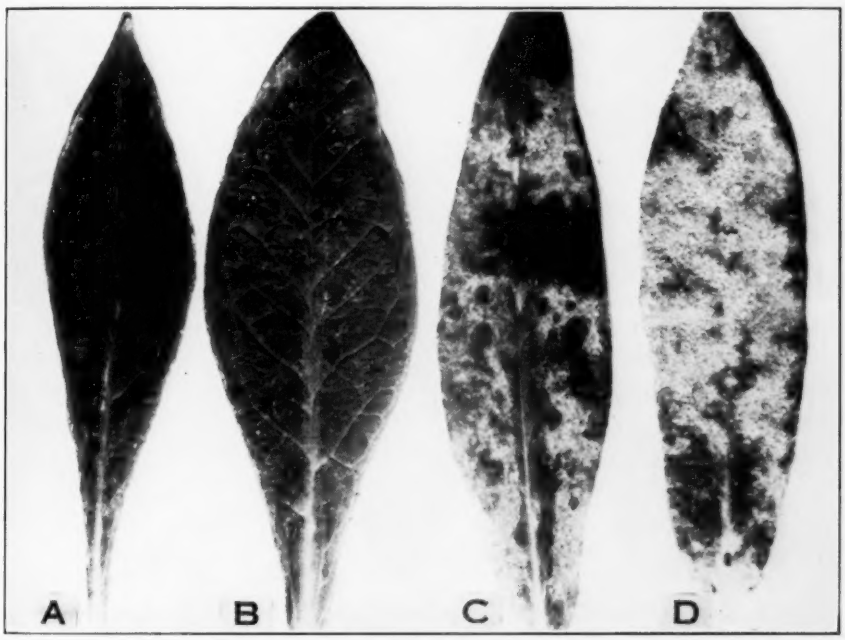

FIG. 1. Leaves illustrating reactions of selections and varieties of perennial phlox to powdery mildew, as the result of inoculations in a greenhouse in 1934. A. Selection H 1, very resistant (compare Fig. 2, B). B. Selection BP 19, very resistant (compare Fig. 2, C). C. Columbia, moderately susceptible. D. Palladin, very susceptible.

susceptible varieties and selections of previous years became heavily mildewed. Moderately susceptible and moderately resistant varieties showed less mildew and the very resistant selections of previous years continued to be so.

In 1938, mildew was again prevalent, commencing early and developing rapidly on susceptible strains. Important changes in reaction occurred. All the original resistant selections developed some mildew. The development was slow and only a moderate infection was reached. They were classified as moderately resistant; however, the differences in reactions,

when contrasted with the freedom from mildew of previous seasons, were pronounced. In 1939 and 1940, red spider was so severe in the planting that mildew reaction could not be determined accurately.

In 1941, mildew again appeared early and developed rapidly; red spider was not troublesome. The varieties and selections were subjected to a severe test with very interesting results. Several of the original highly resistant selections (DP 17, 19, 20) were heavily mildewed (Fig. 2, C). Others (H 1, DP 22, 24) were moderately resistant, as in 1938 (Fig. 2, B). Several (DP 4, 24) showed a high resistance (Fig. 2, A). The results for a selected num-

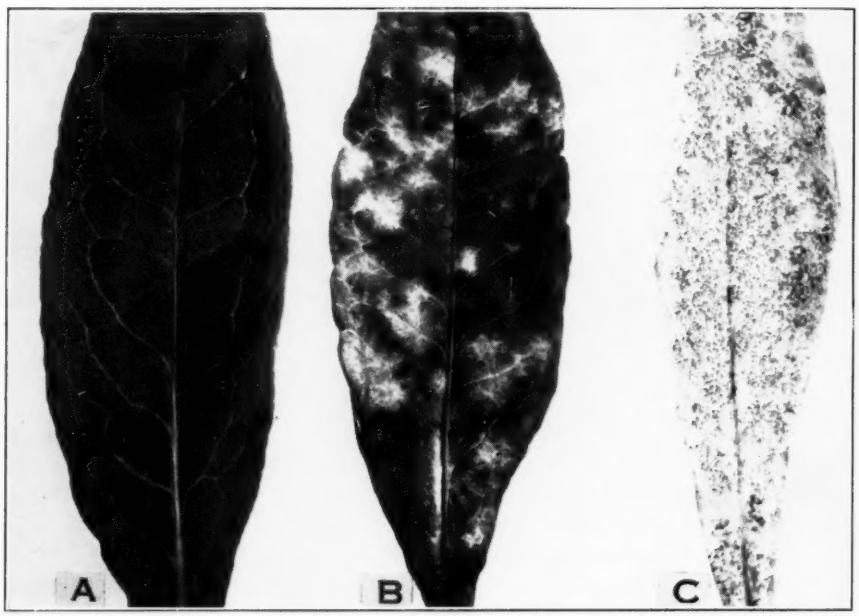

FIG. 2. Leaves illustrating reactions of selections of perennial phlox to powdery mildew in field plantings in 1941. A. Selection DP 4, very resistant. B. Selection H 1, moderately resistant (compare Fig. 1, A). C. Selection BP 19, very susceptible (compare Fig. 1, B).

ber of the selections and varieties are summarized in table 1. The results of the 1938 and 1941 tests strongly indicate that several physiologic races of the mildew fungus occur.

Although no selection has remained entirely mildew-free throughout this study, several have been more or less resistant. Selection is being continued, with promising results. It should be noted that resistant plants in these studies were exposed constantly to inoculation from adjacent heavily mildewed plants. Much more mildew developed on the resistant plants than would have occurred had they been isolated. In the latter case, whenever the resistant plant became infected through wind-borne spores, the resistance of the selection would have reduced the production of inoculum and thereby

greatly retarded the development of the disease. For garden purposes, therefore, the moderately resistant varieties should prove very satisfactory provided susceptible varieties also are not grown.

A number of the resistant selections have mallow purple (Ridgway)

TABLE 1.—*Reactions of a selected number of selections and varieties of perennial phlox to powdery mildew*

Selection or variety	Reaction in[a]				
	1934	1935	1937	1938	1941
H 1	0	0	0	2	2
DP 4	0	0	0	2+	0
DP 17	1	1	0	2	4
DP 19	0	0	0	2	4
DP 20	0	0	0	3	4
DP 22	0	0	1	2	2
DP 24	0	0	0	2	1
Miss Lingard	4	4	4	3	.....
Columbia	3	3	3	4	3

[a] 0 = very resistant, no mildew or only a trace.
1 = resistant, infection scattered and poorly developed.
2 = moderately resistant, development slow and the maximum a moderate amount.
3 = moderately susceptible, development slow, the plants finally well covered.
4 = very susceptible, development rapid, the plants early well covered.
1934 results from inoculation in greenhouse.
1935, 1937, 1938, 1941 results from field plantings.

flowers, including the original selection which is a very vigorous grower, attaining a height of 64 in. under favorable conditions. Several have well-arranged, pure white flowers of good size. Others have white flowers with deep pink "eyes." Some of the recent selections are deep pinks of good quality. There is also considerable range in height of plants and dates of blossoming. The occurrence of physiologic races of the pathogen complicates the problem of obtaining mildew-resistant varieties of phlox. The results, however, thus far secured indicate that varieties of various types can be obtained with considerable general resistance.

ANNUAL PHLOX

In 1935 the study was extended to include annual phlox. In order that the chances for the discovery of resistant individuals might be as great as possible, mixed seed of various types was purchased. From this, 606 plants were grown in a greenhouse and inoculated with powdery mildew. Of these, 480 were classed as susceptible, 68 as moderately susceptible, and 58 as moderately resistant. Seed was obtained from 35 of the moderately resistant plants and plants grown and inoculated in 1936. Of these, 9 lines were outstanding. From these, 156 selections were made and seed obtained. The progenies of these were inoculated in the greenhouse in 1937 and 8 lines were found uniformly highly resistant. Of these, Strain No. 67–25 is the

most promising. In 1941, 91 plants of this line were inoculated in the greenhouse with mildew; all were highly resistant (0-trace). In comparison 168 plants of a commercial strain were susceptible (3–4). Strain 67–25 has large flowers, with a color between pomegranate purple and rose-red (Ridgway) with a large white eye.

SUMMARY

Selections of perennial phlox have been obtained that are highly resistant to powdery mildew. These include a number of color types.

Through repeated selection in annual phlox several lines have been obtained that are highly resistant to powdery mildew.

Field evidence indicates that several physiologic races occur in the mildew differentiated by the reactions of selections of perennial phlox.

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USE OF ERADICANT SPRAYS FOR THE CONTROL OF ASPARAGUS RUST

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INTRODUCTION

Development of rust-resistant varieties of asparagus has been considered a solution of the rust problem in America; consequently, little attention has been given to the control of this disease by other means since the introduction of the Mary and Martha Washington varieties. Resistance in these varieties, however, was known to be near the margin of safety, since rust would often appear in a mild form under conditions favorable for its development. Furthermore, according to recent correspondence with Lawrence Ogilvie[1] of the Long Ashton Research Station, University of Bristol, British and German pathologists consider these varieties only moderately resistant.

During the last 4 or 5 years asparagus growers in Illinois have become alarmed at the amount of rust developing in their fields. This condition has been noted especially in areas of large production. Most of these growers have made an effort to establish their beds from seed or plants certified to be of the Washington varieties. Attempts to control the rust by the use of sprays and dusts have proved ineffective or injurious.

Knowledge of the life cycle of *Puccinia asparagi* DC. led the writers to believe that it might be possible to control the disease by the use of eradicant sprays. There is no good evidence that the rust is perennial, and the only method of initial infection in the spring is from the sporidia of the overwintering teliospores. An efficient eradicant spray, applied during the dormant season, should prevent germination of the teliospores and thus eliminate spring infection. Control under these conditions probably would be local, since a focus of aecial development in the neighborhood of the protected field would soon bring about a general infection from the repeating uredinial stage of the rust. However, it is the opinion of experienced growers that late summer infection does not reduce growth to any extent and, consequently, is of little importance, while early infection results in severe stunting.

The problem of control is simplified in commercial plantings by the fact that in established beds cutting continues until after the period of primary infection by basidiospores. Thus aecia never appear on the plants in harvested fields in northern Illinois, where all the stalks are cut up to the last of June. Young beds left either uncut or cut for only 2 weeks are exposed to the initial infection and thus furnish inoculum for neighboring beds after cutting is discontinued. The main problem, therefore, is to

[1] Letter to H. W. Anderson, dated March 9, 1940.

control the rust on these unharvested fields in order to prevent or delay secondary infection.

The general problem of asparagus-rust epidemiology and control is being reinvestigated by one of the writers (J. P. Fulton). One phase of this problem, the use of eradicant sprays, is here reported, since significant results were obtained that may be of use to other investigators.

PLAN OF THE EXPERIMENT

At Urbana, the plots were located in a field of asparagus that had had a record of heavy rust infection for the past 3 years. Conditions were ideal for secondary infection, since the experimental work on production was based on planting 3 to 4 rows each year and the new plantings were left uncut for 2 years. Thus the harvested rows were always exposed to secondary infection from the new plantings, which always developed abundant aecial pustules. The area selected consisted of 22 rows approximately 250 feet long ranging in age from 3 to 9 years. Rust was evenly distributed over these plots during the summer of 1940, although no exact record of infection was made. Telial material could be collected at any point in the field during the winter months.

TABLE 1.—*Summary of treatment and results of Elgetol eradicant spray experiments at Urbana, Illinois*

Plot[a]	Cultural[b] treatment	Concentration of Elgetol	Dosage per acre	Time of application	Stalks in[c] 50 hills showing aecia June 30	Type of[d] infection
No.		*Per cent*	*Gal.*		*No.*	
1	Uncut	1	800	12/ 5/40	1	L
2	"	1	800	3/27/41	4	L
3	"	None	None	.....	68	H
4	"	2	800	12/ 5/40	47	L–16 H–31
5	Cut	2	800	12/ 5/40	22	L
6	"	1	800	12/ 5/40	12	L
7	"	2	800	3/27/41	8	L
8	"	1	800	3/27/41	7	L
9	"	1	400	3/27/41	8	L
10	"	½	400	3/27/41	2	L

[a] Plots, except 2 and 3, were 25 feet wide, running across 22 rows; Plots 2 and 3 were 50 feet wide, across 11 rows.

[b] "Cut" means old plants were cut off and burned prior to treatment; "Uncut" means that old plants were left until after the spring application.

[c] Fifty hills represents about 300 stalks.

[d] L = light, 1–5 pustules per stalk; H = heavy, 6 or more pustules per stalk.

The material used as an eradicant spray was Elgetol (sodium dinitro-o-cresylate). The plot arrangement, concentration of Elgetol, and date of application are given in table 1. A power sprayer with a Bean Spraymaster gun was used, and the pressure was maintained at 400 lb.

A quiet day was selected for both applications, so that there was little

drift. As indicated in the table, in some of the plots the old plants were removed before application. This was done a few days before the December spraying, when the stalks were thoroughly dry. The plants were raked together and burned, but many stubs from 4 to 12 in. high were left, and abundant telial pustules were observed on these and on débris ("needles" and small branches scattered over the surface of the ground). The object of cutting off the old plants was to facilitate spraying, rather than to eliminate the source of inoculum.

Dosage was based on previous experience with ground sprays in apple orchards. A very thorough wetting of the surface can be obtained at 400 gal. per acre, when the old plants are removed; but, when present, 600 to 800 gal. per acre are required to thoroughly soak all the standing plants and to cover the ground.

The concentrations employed were selected on the basis of previous laboratory and field work on apple scab. The dates of application were selected on the basis of cultural practices, which need not be explained at this time.

In the spring a portion of each plot was left uncut. The remainder was harvested until after initial infection has ceased.

A somewhat similar experiment on a much larger scale was conducted in northern Illinois. The results of these experiments will be published later, but the data secured show a remarkably close correlation with those at Urbana.

RESULTS

Microscopic examination of the teliospores a few weeks after the spring treatment revealed typical collapsed protoplasm, such as had previouly been observed in other fungi. A few spores in each sorus appeared normal, and some sori had as many as 10 per cent of what appeared to be normal spores. Data on germination of the normal and treated teliospores were collected, but will not be presented at this time.

The first aecial pustules were observed during the first week in May. April was dry, excepting the 15th to 20th, when a very heavy rain occurred. Shoots were tagged as they appeared above ground, and, judging from the infection records on these, there was only one extensive infection period, which must have occurred shortly after this rainy period. A 2-inch rain on May 6 apparently did not result in renewed infection, although weather conditions seemed ideal. A like limited infection period prevailed at Rochelle, where the larger experiments were conducted. Because of the limited number of aecial pustules this season, the results obtained from the eradicant sprays are not so conclusive as could be desired.

Records on the number of stalks showing aecial pustules were taken at various times during the spring. A final count on June 30 is recorded in table 1, and will serve as a basis for comparison. It is evident that all of the treatments gave considerable reduction in infection, and that, with the

exception of plot 4, there would appear to be no great difference in the effectiveness of the treatments regardless of concentration, dosage, time of application and previous treatment of the old plants. The contrast between the check and treated plots was especially noticeable when one examined the number of infections (pustules) per stalk. In the treated plots infections usually consisted of 1 or 2 pustules per stalk, while in the check plot nearly every stalk showed numerous pustules, indicating multiple infection.

Plot 4, in spite of a very excellent treatment, shows much higher infection than the other treated plots. Evidently this was due to its being located next to and north of the check plot. This conclusion was further supported by the fact that most of the infection on this plot was at the south end immediately adjoining the check plot.

Observations were made on the spread of the rust as evidenced by the development of the uredia. The data on this phase of the work will be presented in a future publication, but, in general, it was evident that by June 30 the uredia were developing very extensively over the check plot, but were limited to a few areas in the treated plots near the stalks showing aecia, or adjoining the check plot. By July 15 one could see the outline of the check plot at a distance, due to the prevalence of brown plants, while in the treated plots only an occasional infected area could be observed. Adjoining the plots were several rows which had been harvested in April and May, but had been allowed to grow after the cutting season. Plants in these rows were becoming heavily rusted where they adjoined the check plot, but no rust was found on those adjoining the treated plot on July 15.

DISCUSSION AND CONCLUSIONS

An eradicant spray, to be effective against asparagus rust, must reduce teliosporic inoculum to such an extent that few aecial pustules develop, since secondary infections from aeciospores and urediospores build up rapidly. The limited results obtained this season indicate that such a reduction may be secured by the application of Elgetol at concentrations of from $\frac{1}{2}$ to 2 per cent and dosages from 400 to 800 gal. per acre during the dormant season. There seems little difference as to efficiency of fall or spring applications, and the time selected should be governed by the cultural practices of the grower.

It is recognized that a problem exists in areas where large plantings of asparagus are near escaped plants or small garden plantings that are not cut during the early part of the season. Even under such conditions, secondary infection is delayed and is frequently local.

Progressive commercial asparagus growers have abandoned the practice of burning off their beds, since they wish to conserve the humus. Futhermore, burning is not effective in destroying all the inoculum, since observations prove that stubs often are left and the infected needles, which drop

to the ground during the growing season, often are protected during the burning of the bower. The application of an eradicant spray allows the grower to retain the humus-building plants without danger of increasing infection.

Treatment may be confined to young beds, which are not to be harvested the following spring. Under such conditions the cost of the eradicant spray program is not excessive, since, usually, only a small proportion of the total acreage is in young beds.

It is recognized that the ultimate solution of the rust problem in asparagus is the development of more highly resistant strains of our present rust-resistant varieties; but, while awaiting this development, an effective and economical control is believed to have been discovered in the use of an eradicant spray.

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AN ELSINOË CAUSING AN ANTHRACNOSE OF VIRGINIA CREEPER

ANNA E. JENKINS AND A. A. BITANCOURT[1]

(Accepted for publication August 7, 1941)

The presence of an *Elsinoë* on Virginia Creeper (*Parthenocissus quinquefolia* (L.) Planch.) has recently been shown through the discovery of a fungus of this genus on severely diseased leaves, stems, and fruits of this vine growing as an ornamental in the vicinity of Marlboro, New Hampshire.

On August 31, 1940, representative specimens from this source were sent for diagnosis to the Forestry and Recreation Department of New Hampshire, whence they were referred to the Laboratory of the Division of Forest Insect Investigations, U. S. Department of Agriculture, at New Haven, Connecticut, and next, to Alma M. Waterman at the New Haven headquarters of the Division of Forest Pathology of the same department.

Numerous small lesions were present on leaves, stems, and fruit. On discovering that an ascomycete, apparently an *Elsinoë*,[2] was fruiting abundantly on the leaf lesions, Dr. Waterman forwarded the material to the senior writer for further study. The identification was confirmed, as recently noted by Dr. Waterman.[3]

Following the receipt of the mature material from New Hampshire (Fig. 1, A and B), an examination was made of phanerogamic specimens of Virginia Creeper in the U. S. National Herbarium. Abundant infection by the *Elsinoë* was present on a specimen from Florida bearing the date July, 1891. This was from the Chapman Herbarium, having been collected by Dr. Chapman at Apalachicola, where he lived[4] during the latter part of his life.

The *Elsinoë* on Virginia creeper, a new host, is here described as new under the proposed name of *E. parthenocissi.*

Leaf spots few to numerous, generally originating on the upper leaf surface where they are the more distinct, consisting of well delimited circular to subcircular or occasionally irregular areas 0.2–4 mm. in diam., scattered or often distributed along the midrib and veins, or sometimes involving them, where numerous, often forming a more or less coalescent row of lesions serving to outline part or all of the more prominent venation, on dry specimens individual lesions generally depressed below and raised above, with

[1] Paper presented by the senior writer on June 25, 1941, at Windsor, Connecticut, during a session of the summer meeting of The American Phytopathological Society held jointly with the New England branch.

[2] Jenkins, A. E., and A. A. Bitancourt. Revised descriptions of the genera *Elsinoë* and *Sphaceloma.* Mycologia 33: 338–340. 1941.

[3] Waterman, A. M. Diseases of shade and ornamental trees: annotated list of specimens received in 1940 at the New Haven Office, Division of Forest Pathology, Bureau of Plant Industry. Plant Dis. Rptr. 25: 181–186. 1941.

[4] Mohr, C. Alvin Wentworth Chapman. Bot. Gaz. 27: 473–478. 1899.

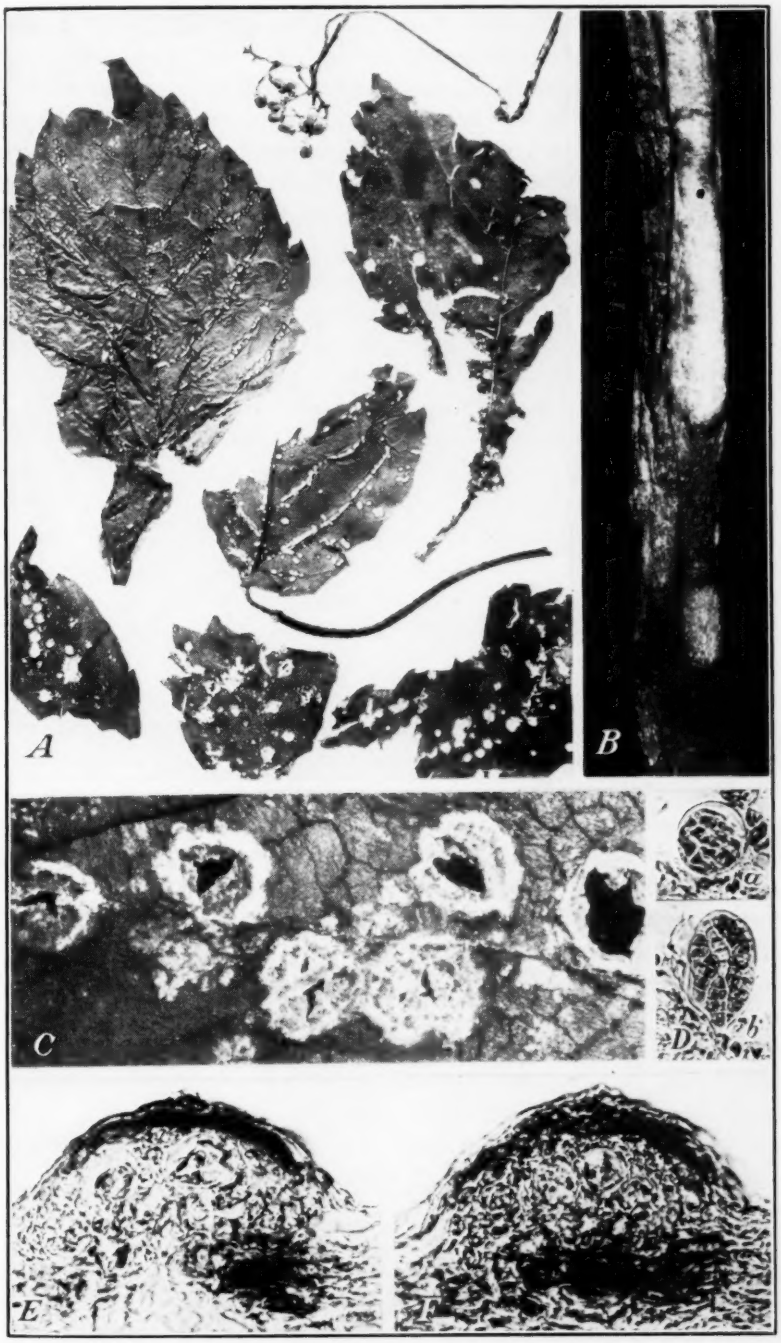

FIG. 1. A. *Elsinoë parthenocissi* on *Parthenocissus quinquefolia*, Marlboro, N. H., Aug. 31, 1940, Mrs. P. S. Howe (type). ×1. B. Stem cankers from lower part of fruiting shoot shown in A. ×10. C. Enlargement of leaf spots, along a vein, upper surface, showing dark fructifications of the *Elsinoë*. ×12½. D. Two asci (a and b) containing ascospores, outer inelastic wall of the ascus in *b*, ruptured and thicker elastic inner wall somewhat expanded. ×500. E and F. Two ascomata showing asci and dark epithecium. ×500. Photographs by M. L. F. Foubert (A–C) and by A. A. Bitancourt (D–F).

generally flattened "vinaceous buff"[5] surface surrounded by a narrow "clove-brown" margin, spots sometimes traversed by prominent fine dark lines representing the smaller venation, part or all of the lesion easily falling away, leaving a shot-hole effect; petiole and stem cankers more or less raised, usually with dark margins and lighter-colored centers; lesions on fruits grayish-white (Waterman); disk also infected; ascomata abundant on the necrotic spots in living leaves amphigenous but more commonly above, at first, intraepidermal; later, penetrating more deeply in the leaf tissues, composed of a hyaline or slightly yellowish pseudoparenchyma of isodiametric, somewhat thick-walled cells; ascomata 70–160 μ in diam., and 35–80 μ in thickness; asci mostly globose, irregularly embedded in the pseudoparenchyma, with thin outer wall, and thick inner wall, particularly at the apex, 17–25 μ in diam., containing 8 ascospores; ascospores 3-septate, constricted at the septae, especially at the median septum, occasionally with a longitudinal septum in one of the middle cells, straight or somewhat curved, with the upper cells broader and shorter than the lower ones, 12–17 by 6–8 μ. Ascospores larger than those of *Elsinoë ampelina* (de Bary) Shear[6] on *Vitis*, which, besides *E. viticola*, is the only other species known on Vitaceae, and is represented only by a single discovery on overwintered cankers on Niagara grape. Additional material of these fungi will be necessary for an adequate comparison of them.

Maculae in foliis e parcis numerosae, supra conspicuores, bene definitae, generaliter circulares vel subcirculares, 0.2–4 mm. in diam., sparsae vel per nervum medium et nervos alteros dispositae, in foliis vinaceo-alutaceae, brunneo-marginatae, in fructibus griseo-albae; cancri in petiolis caulibusque plus minusve elevati margine fusco et centro pallidiore; ascomata in maculis foliorum vivorum abundantia, amphigena, supra numerosioria, prominentia, ascomata 70–160 μ in diam. et 35–80 μ crasso; asci plerumque globosi, in parenchymate hyalina vel pallide flavidula irregulariter immersi, 17–25 μ in diam.; ascosporae 3-septatae, ad septa praecipue medio constrictae, cellula media una interdum longitudinaliter septata, rectae vel subcurvatae, cellulis superioribus latioribus et brevioribus, 12–17 μ longis, 6–8 μ latis.

On leaves, stems and fruit of *Parthenocissus quinquefolia* (L.) Planch. (= *Ampelopsis quinquefolia* (L.) Michx. and *Psedera quinquefolia* (L.) Greene), Marlboro, New Hampshire, and Apalachicola, Florida, causing a destructive anthracnose, here so designated in accordance with this term as originally[7] applied to the equivalent disease of grape.

[5] Colors in quotations are based on Ridgway, R., Color Standards and Color Nomenclature, 43 pp. and 53 colored plates (Washington). 1912.

[6] Shear, C. L. Life history of *Sphaceloma ampelinum*. Phytopath. **19**: 673–679. 1929. 1929.

[7] Fabre, E., and Dunal, F. Observations sur les maladies régnantes de la vigne. Bul. Soc. Cent. d'Agr. Dép. de l'Hérault **40**: 11–75. 1853.

Specimens examined:

Apalachicola, Fla., July 1891, A. W. Chapman. (Mycological Collections, Bureau of Plant Industry 73808. This fragment of the phanerogamic specimen from the Chapman Herbarium now filed in the U. S. National Herbarium under accession number 958621, was obtained through the courtesy of W. R. Maxon.)

Marlboro, N. H., Aug. 31, 1940. Mrs. P. S. Howe (type) (Myc. Coll., B.P.I. 73616. Phytopathological Section Instituto Biologico, São Paulo 4022) June 28, 1941, A. E. Jenkins; July 29, 1941, Mrs. P. S. Howe.

BUREAU OF PLANT INDUSTRY,
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ROUGH-BARK, A VIRUS DISEASE OF FLOWERING CHERRY[1]

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(Accepted for publication September 2, 1941)

An abnormal growth condition of the Kwanzan variety of flowering cherry (*Prunus serrulata* var. *Kwanzan*) was called to our attention in 1939. In a nursery planting the trees were dwarfed in height and seldom produced any lateral branches. The bark, instead of being smooth and a normal gray, was deep brown and roughened by longitudinal splitting. The leaves were grouped close together due to a shortening of the internodes, and most of the leaves were arched downward by the improper development of the midrib (Fig. 1, B). These clustered heads of foliage and the curling of the leaves are similar to the effects of aphid colonies on cherry leaves. There is often a longitudinal splitting and cross cracking of the under surface of the midribs of the leaves. Vein clearing and ragged leaves formed by the cutting out of necrotic areas on the young leaves are prominent symptoms on rough-bark trees, but these symptoms are likewise present on most varieties of *Prunus serrulata* that are not affected with the rough-bark condition. This vein-clearing and ragged-leaf condition may prove to be due to another virus or to physiological conditions.

Similar rough-bark symptoms have been observed on two large Kwanzan trees. The leaf symptoms and splitting of the bark of the younger wood is similar to that noted on the nursery trees, but on the older wood the splitting and roughening of the bark are more pronounced (Fig. 1, A). The seasonal growth of these trees is from 6 to 12 inches long, while that of the normal trees is 2 to 3 feet. The symptoms of these trees immediately suggested that the trouble might be of virous origin.

In the nursery where the disease was first observed, there were 592 trees affected with this condition in a planting of 3,887 trees. The trees were grouped in row areas consisting of 8 or 10 consecutive trees, or approximately the same number of trees as the average number of buds obtained from one bud stick. This segregation of abnormal trees suggested a bud-perpetuated disease, and an examination of the mother block where the buds had been taken showed 2 trees with symptoms similar to those of the young trees. The following experiments were conducted to determine the nature of this trouble.

A block of 75 mazzard seedlings, which are commonly used as root-stock for flowering cherries, was divided into 3 lots of 25 trees each. One lot was budded with buds taken from a normal Kwanzan tree; another lot was budded with buds taken from a rough-bark Kwanzan tree; and the third was budded with buds taken from both the healthy and diseased trees. In

[1] Published as Technical Paper No. 388 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Botany Department.

the latter, the normal bud was introduced near the soil line and the bud from a tree showing rough-bark symptoms was placed on the same seedling but on the opposite side of the stem and 12 inches above the normal bud. The trees were allowed to develop into 2-year-old nursery trees before the final observations were made. The lot budded with normal buds produced

FIG. 1. Rough-bark of Kwanzan flowering cherry. A, Branch from large diseased tree showing characteristic bark symptoms. B, Illustrates rough-bark of stems and downward arching of the leaves on a 2-year-old nursery tree.

22 well-developed normal trees. The lot budded with rough-bark buds produced only 6 trees, and these were dwarfed and showed typical rough-bark symptoms. Where healthy and diseased buds were placed on the same seedling, both buds developed on 7 of the trees, only the normal bud devel-

oped on 9 other trees, and only the rough-bark bud developed on 3 trees. All growth that resulted from these buds showed characteristic rough-bark symptoms regardless of the type of bud used or its position on the seedling. Of special interest is the group of 9 rough-bark trees produced by the normal bud, even though the rough-bark bud did not develop. This would indicate that the virus passed into the mazzard seedlings from the rough-bark bud, even though organic union was not sufficient for bud development. This was demonstrated again the following year when 24 mazzard seedlings were budded with rough-bark in October. When these buds failed to develop, the same seedlings were grafted with scions from normal trees the following March. Of the 24 trees that developed from the scion wood, 23 showed typical rough-bark symptoms, while adjacent seedlings that had not been previously budded with the rough-bark buds, but were grafted with scion wood from the healthy tree, grew into normal trees. In many instances new shoots of the mazzard seedling have developed from the roots of Kwanzan trees showing the disease, but these sprouts did not show rough-bark symptoms. This indicates the mazzard seedling may become a symptomless carrier of the virus.

These observations and transmission experiments suggest that a virus is concerned. The symptoms produced by the virus on this host are very characteristic and unlike any of the other known viruses of *Prunus*. The common name, rough-bark, is offered for this new disease, and, in the number system, *Prunus virus 9* may be assigned to the virus. For those using Holmes' system of nomenclature, both a new genus and species name is proposed. The genus name of *Rimocortius* is based on the most characteristic symptom of the disease, *bark cracking*. Since the disease seems to be limited to the Kwanzan variety, the name **Rimocortius Kwanzani** is assigned to it.

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MALE-STERILE BARLEY FOR STUDY OF FLORAL INFECTION[1]

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(Accepted for publication August 27, 1941)

A male-sterile barley, suggested as a new tool for the plant breeder,[3] also appears to provide a means for mass inoculation of barley flowers with cultures of disease-producing organisms transmitted by floral infection. The procedure should be particularly useful in studies of conditions affecting infection. At present such studies would necessarily be limited to the few varieties possessing the male-sterile character, but there seems to be no reason to assume this character may not be found in or introduced into other varieties as needed. The male-sterile plants have been shown to be self-sterile, and highly cross-fertile. Ratios of 3 fertile : 1 male-sterile in F_2 populations, and 1 fertile : 1 male-sterile in backcrossed populations demonstrate the simple inheritance of the character. Of particular interest to plant pathologists are the open glumes of the male-sterile plants, which permit rapid inoculation. The degree and persistence of glume opening and of cross fertility have been observed to vary somewhat with the environment. Satisfactory seed sets commonly result from natural crossing and very good seed sets have been obtained with controlled pollination.

Male-sterile barley spikes were inoculated with spores of the organisms causing barley stripe (*Helminthosporium gramineum* Rabh.), which disease is fairly common in California, and of loose smut (*Ustilago nuda* (Jens.) K. & S.), which seldom persists in California, probably because of the dry atmosphere.

In the stripe-infection experiments (Table 1), male-sterile spikes were first pollinated by a mass dusting of the spike with pollen from F_1 hybrid plants that were heterozygous for the male-sterile factor. Then, leaves collected from plants killed by stripe were pulled from the top of the spike downward between each row of glumes to facilitate discharge of spores into the open florets. The inoculated spikes were then bagged. Percentages of infection obtained on the 3 spikes treated were satisfactory, as shown in table 1, and were about equal to any obtained at this station, by the culture of sprouting barley on a medium-base supporting mycelial growth of the stripe organism.

The tests with loose smut involved mass dusting with both pollen and smut spores. Infections of loose smut (Table 1) were rather low. This

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[2] Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; and Instructor in Plant Pathology and Junior Plant Pathologist in the Experiment Station, respectively.

[3] Suneson, C. A. A male sterile character in barley. Jour. Hered. 31: 213–214. 1940.

TABLE 1.—*Infections obtained with the barley diseases, loose smut (Ustilago nuda) and stripe (Helminthosporium gramineum), by floral inoculation of mass-pollinated male-sterile barley plants at Davis, California*

Pollen parents (F_1 hybrids) used on male-sterile plants	Seeds set on spike	Plants established		
		Healthy	Infected	
	Number	*Number*	*Number*	*Per cent*
Loose smut:				
Male-sterile × C.I. 5636	27	15	3	16.7
Male-sterile × *nudeficiens*	33	26	1	3.7
Do	32	21	1	4.5
Do	33	20	1	4.8
Do	25	17	2	10.5
Male-sterile × Meloy	30	22	0	0.0
Male-sterile × *nigrinudum*	43	28	2	6.7
Do	57	26	12	31.6
Do	20	10	2	16.7
Do	50	34	6	15.0
Stripe:				
Male-sterile × Colsess V	45	2	17	89.5
Male-sterile × *nigrinudum*	52	12	28	70.0
Do	33	5	25	83.3

may have been due to varietal resistance, low atmospheric humidity at the time of infection, or to poor viability of the smut spores. Tapke[4] showed that the viability of loose smut spores is soon lost. The plants from which the smut was collected were headed on April 4, but the smut was not collected and used until April 10 to 17, when most of the spores had been blown away. Among the 30 smutted plants were 5 that produced some disease-free spikes, of which 1 had normal fertility and 4 were male-sterile.

[4] Tapke, V. F. A technique for identifying the loose smuts of barley. Phytopath. 31: 284–286. 1941.

PHYTOPATHOLOGICAL NOTES

A Chemical Method for the Determination of Tobacco-mosaic-virus Protein in Plant Extracts.—Biological assay methods for measuring virus activity are subject to a rather large error. It is impossible to convert virus activity, thus measured, into quantity of virus protein per cc. of extract, even when comparing an unknown against a purified virus preparation of known virus protein content. This is due to variation in the state of aggregation of chemically purified virus preparations and to other factors relating to the plants and process of inoculation.

Martin, Balls, and McKinney[1] described a method for measuring virus protein in tobacco leaf tissue based upon the observation that proteins of healthy tobacco leaves are partly digested by trypsin, whereas the virus protein resists such digestion. About 20 per cent of the nitrogen of leaf tissue of healthy plants was not rendered soluble by tryptic digestion. Because this undigested portion was several times larger than the quantity of virus protein, it appeared likely that this may have introduced an appreciable error in the results.

This problem was studied further, using extracts of healthy and mosaic-diseased leaves in order to eliminate the large fraction of insoluble tissue nitrogen shown incompletely digested by trypsin. Trypsin digestion, when applied to leaf-tissue extracts, left the proteins of healthy tobacco leaf extracts incompletely digested in 24 hours at pH 7.5 and 37° C. The quantity of trypsin-resistant protein varied from 23 to 54 per cent of the total soluble protein, depending upon source of material. Increasing the trypsin concentration from 1 to 5 mg. per cc. of extract did not reduce the amount of undigested protein.

Several methods were studied for separating virus from non-virus proteins of tobacco leaf extracts, including thermal fractionation and use of various protein precipitants and denaturants. The method finally adopted is based upon the observation by Best[2] that tobacco mosaic virus is precipitated from aqueous solutions at its isoelectric point, pH 3.4.

An extract of mosaic-diseased tissue was prepared by adding 1 cc. of M/10 phosphate buffer, pH 7.0, per g. of chopped frozen tissue. After thawing, the tissue extract was pressed out and clarified either by centrifuging or filtering through "Celite." A 40-cc. portion of the clear extract was measured into a 50-cc. centrifuge tube and acidified to pH 4.2 to 4.0 by cautiously adding N/10 H_2SO_4. A large portion of the non-virus protein precipitated during overnight refrigeration was removed by centrifuging at 3000 r.p.m. The supernatant liquid was decanted, further acidified to pH 3.4, allowed to refrigerate overnight, and then centrifuged 30 minutes at 3000 r.p.m. The precipitate was transferred to a 10-cc. centrifuge tube and protein nitrogen was determined by precipitation with 2.5 per cent

[1] Martin, L. F., A. K. Balls, and H. H. McKinney. Jour. Biol. Chem. **130**: 687. 1939.
[2] Best, R. J. Austr. Jour. Exper. Biol. and Med. **14**: 1. 1936.

tri-chloro-acetic acid and digestion and distillation as ammonia in a micro-Kjeldahl apparatus. Since the protein precipitated at pH 3.4 contains virus protein plus a trace of non-virus protein, it is necessary to run a blank on an extract of healthy leaf tissue.

Accuracy of the method was determined by measuring the quantity of virus-protein nitrogen in extracts of healthy tobacco tissue amended by known amounts of purified virus protein (Table 1).

TABLE 1.—*Recovery of virus protein added to 30 cc. of leaf extract from healthy tobacco*

Virus N added	Virus N recovered[a]	Virus N recovered
mg.	*mg.*	*percentage*
0.305	0.259	85
0.611	0.553	91
1.020	1.030	101

[a] Blank on 30 cc. healthy leaf extract yielded 0.05 mg. N, which figure was used for correcting the results.

The concentration of virus protein in mosaic-diseased tobacco leaves usually is in the range of 0.6 to 3.0 mg. of virus nitrogen per 40 cc. of leaf extract. Within this range of concentration the error of estimation by the above method is less than 10 per cent.

The virus-nitrogen content of extracts of mosaic-diseased tobacco leaves was determined by the foregoing precipitation method and compared with lesion counts obtained from assays on primary leaves of Scotia beans. Comparisons were made against a control inoculated on one leaf of each pair of bean leaves (Table 2).

TABLE 2.—*Comparison of lesion counts and virus nitrogen content of extracts of mosaic tobacco leaves*

Soln.	Virus N per 40 cc. extract	Virus assay[a]		
		Solution (10^{-3})	Control[b]	Per cent of control
	mg.	*lesions/leaf*	*lesions/leaf*	
I	2.23	33.7	74.6	45.17
II	2.04	20.1	59.2	33.95
III	1.53	17.3	54.4	31.80

[a] 30 Scotia bean leaves inoculated with each solution.
[b] Control was identical for each set of 30 plants, and served to facilitate comparisons between the three solutions on a percentage basis.

The isoelectric precipitation method has a smaller experimental error than the biological-assay methods, and can replace them in certain work. The method is rapid and permits expression of absolute quantities rather than as comparative lesion counts, affected by aggregation of infective particles, by host susceptibility and other factors. Biological assays, however, must be used when virus activity is in question, when only small amounts

of virus are available, and when the concentration of virus protein is very low.—CLAUDE H. HILLS, Protein and Nutrition Research Division, Bureau of Agricultural Chemistry and Engineering, and H. H. MCKINNEY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Transmissible Rough-bark Diseases of Fruit Trees.—Several attempts have been made in recent years to classify viruses, reaching the extreme of applying a Latin binomial ostensibly based on virus characteristics but actually based exclusively on symptoms of the disease. Parallel with this trend and perhaps to some degree because of it, there seems to have been a decline of interest in the orderly grouping of *diseases* caused by viruses.

Most of the virus diseases of plant that have been studied can be and often are placed in one of two large groups, the mosaic and yellows diseases. There appears now to be a considerable number of virus diseases of woody plants, mostly little known, falling naturally into a third group,[1] type have been described, notably psorosis of citrus,[2] stony pit of pear,[3] and diamond canker of prune.[4] The diseases of this group affect the bark primarily, the wood occasionally, the leaves and fruit infrequently. They are extremely slow in development in the tree. The evidence to date indicates a narrow range of susceptible species or varieties for each disease.[4] Thus, trees affected by a transmissible disease of Bosc pear similar to or identical with stony pit have been top-worked to Bartlett, Comice, and Hardy, with no fruit symptoms on the 3 last named varieties up to 5 years for Bartlett and Hardy and 6 years for Comice from the time of grafting. There are indications, also, of more than one disease of this type in a single fruit species.[2] For example, the Bosc disease just referred to produces in Hardy a mosaic pattern in leaves, but no fruit symptoms, while a similar disease occurring naturally in Hardy in the same district is transmissible by grafting from Hardy to Hardy producing fruit pitting but not clear-cut leaf symptoms.

A considerable number of infection experiments have been initiated but at least several more years will be required to bring most of them to a final conclusion. On the basis of symptoms, distribution in the orchard, persistence in cions, and, in a few cases, transmission by grafting, the following diseases found in central and northern California may be mentioned as probably belonging in the rough-bark group. Diseases of the diamond-canker type occur on apricot, cherry (*P. avium*), plum (*P. domestica*),

[1] Thomas, H. Earl. Graft transmission persistence and migration of some viruses in fruit trees. (Abstract) Phytopath. **30**: 790. 1940.

which may be designated as rough-bark diseases. Several diseases of this

[2] Fawcett, H. S. Psorosis in relation to other virus-like effects on citrus. (Abstract) Phytopath. **29**: 6. 1939.

[3] Kienholz, J. R. Stony pit, a transmissible disease of pears. Phytopath. **29**: 260–267. 1939.

[4] Smith, Ralph E. Transmission of diamond canker of the French prune. Phytopath. **31**: 886–895. 1941.

prune, and rose (Pink Radiance) (Fig. 1, C). All of these persist in cions.[5] A disease of Gravenstein apple (Fig. 1, D), known locally as flat limb, is characterized in the apple chiefly by irregularities in wood development, but may be included here because of the rough-bark symptoms produced (Fig. 1, A) when inoculations are made from apple to *Pyracantha*. At least the two diseases referred to above, and apparently more, are found in pears ranging in bark symptoms from measles on younger branches to

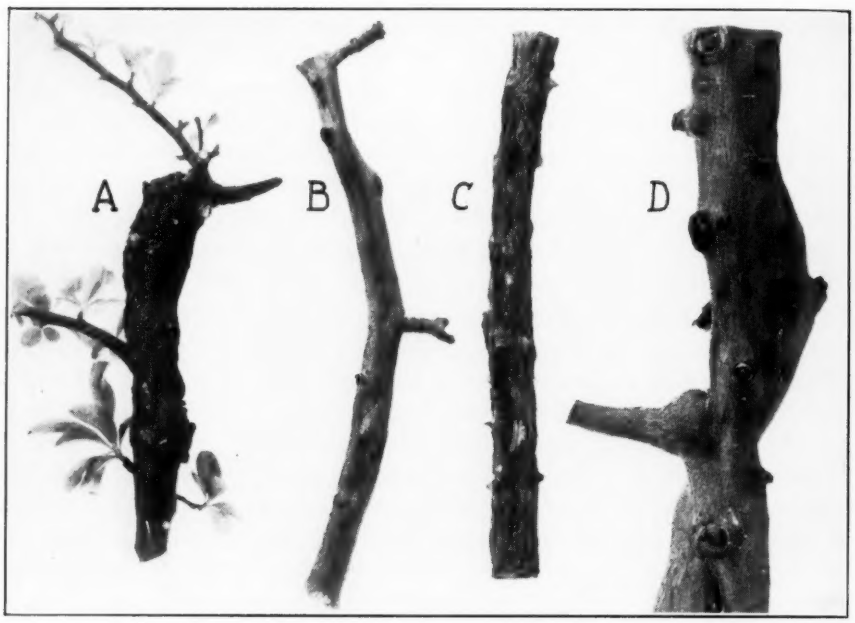

FIG. 1. A. Symptoms produced on *Pyracantha gibbsii yunnanensis* by graft inoculation from apple of the type shown at D. B. Depressions and distortion in Bosc pear associated with "measles" and fruit pitting. C. Pink Radiance rose naturally affected by rough bark condition. D. Gravenstein apple branch affected by "flat limb" or "crinkle-wood."

excessive sloughing of outer bark or "oak bark" on older parts. In addition, the affected Bosc pear may display symptoms (Fig. 1, B) not greatly unlike those of the Gravenstein disease (Fig. 1, D).

Other diseases have been seen on almond, apple, peach, and quince, which may well belong in this group; but the above may suffice to indicate that a very considerable number of diseases and kinds of woody plants are involved in the group.—H. EARL THOMAS, Division of Plant Pathology, University of California, Berkeley.

Two Genetic Characters of Tomato Fruits that Might be Mistaken for Symptoms of Disease.—Broad, dark-green stripes resembling mosaic symptoms were found in the peel of 2 tomato fruits on a plant of (T560) Michi-

[5] H. N. Hansen has collected *Alnus* (*rhombifolia?*) with cankers very similar to those of prune.

gan State Forcing (Ferry-Morse Seed Co.) at the Tomato Disease Laboratory, Jacksonville, Texas, in May, 1939. This abnormality apparently resulted from a mutation. In 1940, seed from these 2 fruits produced 23 plants with many striped fruits. Prominent, broad, dark-green stripes extended from stem to blossom end in the peel of the small green fruits. As the fruits developed to the green-wrap stage, deep pits and grooves formed in the peel of many of the striped fruits. On ripening, the dark-green stripes became yellow in contrast to the normal red part of the fruit (Fig. 1).

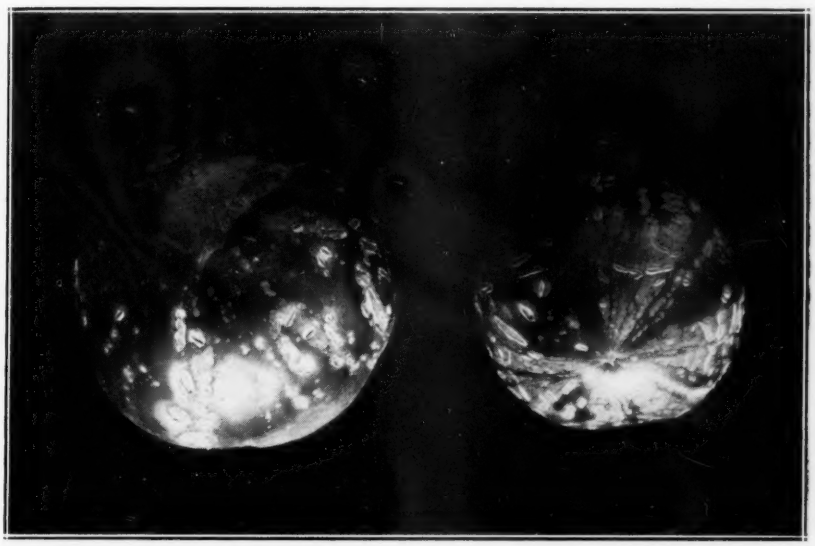

FIG. 1. Stripes, pits, and grooves in tomato-fruit peel of T560. × ½.

Seed from 4 of the striped fruits was tested for a third generation in fields in 1941. The parent fruits of T560B and T560D showed only stripes, while the parent fruits of T560C and T560E bore both stripes and pits. In the fields 72 per cent of the 184 plants of T560B and T560D bore striped and pitted fruits, while 94 per cent of the 221 plants of T560C and T560E produced striped and pitted fruits. These data are now interpreted as indicating inheritance of the stripe and pit characters. Many of the T560 tomato plants bore both striped and normal fruits, and the writer has observed such stripes and pits only in this selection.

None of these plants showed any other abnormality that might be associated with the stripes and pits, and there was no indication that this might be a virus disease. The stripes and pits were much more prominent in T560 than in fruit with tomato fruit pox,[1] which resembles mosaic and is not transmitted by seed. They differed from fruit stripe of tomato,[2] which causes raised light-green to ashy-gray stripes in the peel, associated with

[1] Ivanoff, S. S. and P. A. Young. Tomato fruit pox. Phytopath. **30**: 343–345. 1940.

[2] Jones, L. K. Fruit stripe of tomato caused by a tobacco type I virus. Phytopath. **30**: 538–540. 1940.

mild mosaic of the leaves.—P. A. YOUNG, Tomato Disease Laboratory, Texas Agricultural Experiment Station, Jacksonville, Texas.

Downy Mildew of Lima Beans in Colorado.—In 1889 Thaxter reported that the downy mildew was causing considerable damage to Lima beans in certain regions along the Atlantic Coast and described it as a new species, *Phytophthora phaseoli*. Since then it has been observed to be more or less common in many of the Eastern States and has been reported from as far west as Ohio.

It has been generally assumed that the downy mildew required high humidities for its development, which probably explains why it occurs mostly in the eastern part of the United States and the South, and less frequently in the interior. The fact that it appeared in northeastern Colorado during the summer of 1941 in an epidemic form suggests that either the arid regions sometimes become sufficiently humid for infection, or the downy mildew of Lima beans can adapt itself to arid conditions. During the many years the writers have visited this location each summer, downy mildew has never been previously observed.

Only a few varieties of Lima beans were grown in this locality where the observations were made. In one field each of the Henderson Bush, Woods Prolific, and Jackson Wonder varieties, 100 per cent of the plants were infected and the losses amounted to as much as 60–75 per cent. In one adjoining field the disease could not be found, showing it to be exceedingly spotty. Lima beans are grown entirely by surface irrigation in Colorado. The fungus occurred on the pods in all stages of their development and not on any other organs of the plant. The conidia, measuring somewhat larger than those described by Thaxter, were present in great abundance both on the outside and inside the pods. Zoospores and oogonia were not observed.

The summer of 1941, in Colorado, was unusual in that rains were rather frequent and the nights cool over a long period of time. Dews were frequent and abundant.

Lima bean seed is not grown in the States along the Atlantic Coast, where the disease is most prevalent, but only in Colorado and in the States farther west, whence downy mildew has not been reported, except in California. The disease has not been reported in California for many years, and its occurrence then was probably near the coast, where the humidity was high. In view of these facts it would be of unusual interest to know the source of the initial infection which reached epidemic proportions in Colorado in a single season.—L. L. HARTER AND W. J. ZAUMEYER, U. S. Horticultural Station, Beltsville, Md.

A New Virus Disease of Bean.—A virus disease of bean, tentatively designated as bean-mosaic virus 4, distinctly different from any hitherto described bean virus, was recently isolated from severely mottled pods of several varieties. Two types of symptoms, depending upon the bean variety inoculated, are produced. Local necrotic lesions are produced on some varieties, whereas on others only systemic mottled symptoms are produced.

In no case has any variety thus far tested exhibited both local and systemic infection on the same plant. Evidence shows only a single virus is involved.

The local lesions appear in about 2 to 4 days following inoculation. They are fairly circular, brownish red, and vary from 1 mm. to 3 mm. in diameter. The systemic mottled symptoms appear in about 10 days after inoculation and are not greatly unlike those produced by bean virus 1 on many varieties. In general, those varieties that are somewhat tolerant to bean virus 1 exhibit quite a severe mottling when infected with bean virus 4. The symptoms on the pods are more pronounced than those produced by any virus thus far reported to be infectious to bean. They are characterized by dark-green irregularly shaped, watersoaked-like blotches on some green-podded varieties and by greasy, shiny areas on others. On wax-podded varieties, greenish-yellow areas are noted. Infected pods may be slightly malformed, subnormal in length, and frequently curled at the end due to improper ovule development.

Seventy-nine varieties of beans have been inoculated and all have shown one symptom or the other after inoculation. Thirty-three varieties showed local lesion infection and 46 varieties were systemically infected. None of the varieties showing local lesions exhibited systemic infection, and *vice versa*. Of the 8 varieties known to be immune from bean virus 1, Corbett Refugee, Great Northern U.I. No. 59, and Red Mexican U.I. No. 34, exhibited local lesions when inoculated with bean virus 4. Idaho Refugee was heterozygous for the two symptoms, while U.S. No. 5 Refugee, Sensation Refugee No. 1066 and No. 1071, and Robust manifested mild systemic symptoms. Varieties showing local lesions upon artificial inoculation could be regarded as commercially resistant under field conditions. In tests thus far conducted, the host range of the virus is restricted to the Leguminoseae.

Preliminary studies on the properties of the virus show that the thermal inactivation point lies between 90° and 95° C., which is higher than that for any legume virus thus far described and approximates that of tobacco mosaic. It was infectious at a dilution of 1–500,000 and retained its infectivity after aging for 165 days at 18° C.

Immunological studies have shown no relationship to bean virus 1.

Little is known regarding the distribution of this virus under field conditions. It has been isolated from bean plants grown in Maryland, California, and Louisiana.—W. J. ZAUMEYER and L. L. HARTER, Division of Fruit and Vegetable Crops and Diseases, U. S. Horticultural Station, Beltsville, Md.

Classification and Nomenclature of the Pathogen Causing Bacterial Ring Rot of Potatoes.—In a recent article Thornberry[1] comments on the lack of consistency in citing the authorities for *Phytomonas sepedonica*, but the reason for this is not discussed. The main cause for the confusion appears to be the fact that Spieckermann first used the name *Bacterium sependonicum* in 1913 in a popular agricultural periodical[2] but omitted a description

[1] Thornberry, H. H. Bacterial ring rot of potatoes in Illinois. Plant Dis. Rptr. 25: 509–510. 1941.

of the organism. The name was *nomen nudum* and, therefore, did not constitute true publication. It is this that certain investigators appear to have overlooked. In 1914 Spieckermann and Kotthoff[3] gave a description of the pathogen and other investigators accept the second article as the authentic publication of the species. The writers are of the same opinion. In 1920 E. F. Smith[4] referred to the species as *Aplanobacter sepedonica*, omitting a citation to authors. Further confusion arose in 1937 when Magrou[5] placed the pathogen in the genus *Phytomonas* only 3 months ahead of Savile and Racicot.[6]

American investigators have followed Magrou in the use of the generic name *Phytomonas* for the pathogen, but no discussion has arisen as to the correct position of the species among the other bacteria. Elliott[7] has pointed out the invalidity of the name *Phytomonas;* and Burkholder[8] and Dowson[9] have shown that as the genus exists at present it is composed of a heterogeneous collection of bacteria. The existing species should be allocated to other genera. Jensen,[10] in discussing the genus *Corynebacterium*, states that possibly *Phyt. sepedonica* belongs here. This genus was established in 1896 by Lehmann and Neumann[11] for the diphtheria bacterium, and has been accepted by bacteriologists at large. A thorough study of the morphology and physiology of the ring-rot pathogen has convinced the writers that this organism has all the characteristics of a *Corynebacterium*. The most characteristic features placing it in this category are (1) its pleomorphism including large numbers of club-shape cells, (2) non-motility, (3) V- or L-shape cells indicating "snapping division," (4) its Gram reaction, non-acid fastness, and its uneven staining, and (5) its cells occurring mostly singly, very seldom even in pairs. Physiological properties characteristic of *Corynebacterium* are (1) its demand for a complex medium rich in protein, (2) its strict aerobic growth, (3) its general biochemical inactivity including poor gelatine liquefaction, poor starch hydrolysis, slow action on litmus milk, etc., and (4) its very slow rate of growth on all ordinary media.

On the basis of these major and minor characteristics it is proposed that

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[2] Spieckermann, A. Zur Kenntnis der in Deutschland auf treten den Gefasskrankheiten der Kartoffelplanze. Illus. Landw. Zeitung. **33**: 680–682. 1913.

[3] ———— and P. Kotthoff. Untersuchungen über die Kartoffelpflanze und ihre Krankheiten. I. Die Bacterienringfäule der Kartoffelpflanze. Landw. Jahrb. **46**: 659–732. 1914.

[4] Smith, E. F. Bacterial diseases of plants. p. 207. Saunders, (Philadelphia). 1920.

[5] Magrou, J. *Phytomonas. In* Dictionnaire de bactéeries pathogènes by P. Hauduroy, G. Ehringer, A. Urbain, G. Guillot and J. Magrou. Masson et Cie (Paris). 326–437. 1937.

[6] Savile, D. B. O. and H. N. Racicot. Bacterial wilt and rot of potatoes. Sci. Agr. **17**: 518–522. 1937.

[7] Elliott, Charlotte. The genus *Phytomonas*. Phytopath. **27**: 1181–1182. 1937.

[8] Burkholder, W. H. The taxonomy and nomenclature of the phytopathogenic bacteria. Phytopath. **29**: 128–136. 1939.

[9] Dowson, W. J. On the systematic position and generic names of the Gram-negative bacterial plant pathogens. Zentralb. Bakt. Abt. II. **100**: 177–193. 1939.

[10] Jensen, H. L. Studies on saprophytic Mycobacteria and Corynebacteria. Proc. Linn. Soc. New S. Wales. **59**: 19–62. 1934.

[11] Lehmann, K. B., and R. Neumann. *Corynebacterium*. Atlas und Grundriss der Bakteriologie. Teil. II. 1 Auft. 108 and 350. Lehmann (München). 1896.

the name of the ring-rot organism be changed to **Corynebacterium sepedonicum** (Spieckermann et Kotthoff) comb. nov.—J. B. SKAPTASON and W. H. BURKHOLDER, Department of Plant Pathology, Cornell University, Ithaca, New York.

Scolytus sulcatus and Apple Trees in Relation to the Dutch Elm Disease Control Program.—Scolytus sulcatus LeC., a bark beetle, commonly reported on apple and occasionally on related species of trees in central and eastern New York, western Connecticut, and northern New Jersey, has been reported feeding on and breeding in elm.[1] Recently, *S. sulcatus* has been found capable of transmitting *Ceratostomella ulmi* Buisman under controlled conditions from infected elm logs to nursery elms in cages.[2]

A preliminary investigation of the pathogenicity and longevity of *Ceratostomella ulmi* in apple trees and wood has been conducted during the past 2 years. On June 5, 1939, 100 wild-apple seedlings (*Pyrus malus* L.), 1 to 1½ in. in diameter at the base and 5 to 12 ft. tall, were inoculated at one point near the base of the trunk. Inoculation was effected by making a vertical tangential incision into the xylem with a ⅛-in. wood chisel and then immediately filling the wound with an aqueous suspension of spores of *C. ulmi*. Ten comparable trees were similarly inoculated with sterile water as checks. No external symptoms of the disease were noted on any of the trees. During the first week of September, 1939, 50 of the inoculated trees and 5 of the check trees were cut and examined for infection. Light-brown discoloration, which in 16 cases extended into the small twigs, was observed in the xylem of all of the 50 inoculated trees (Fig. 1). The minimum, maximum, and average spreads of *C. ulmi* from the points of inoculation, as determined by the recovery of *C. ulmi* by culturing, were 5.25, 111.68, and 47.75 inches, respectively. *C. ulmi* could not be isolated from any of the check trees.

After examination the trunks of the 50 inoculated trees were placed outdoors on the ground in dense shade. On September 24, 1939, coremia were noted growing on cut surfaces of 11 of the trunks. The heads from several coremia on each trunk were cultured and in each case proved to be *Ceratostomella ulmi*. On October 1–3, 1940, cultures were made of discolored streaks in the xylem from the 50 trunks that had been stored on the ground approximately 13 months. *C. ulmi* was recovered from 19 of the trunks. These 19 trunks were cultured again on March 5, 1941, but *C. ulmi* was recovered in only 2 cases. Efforts to recover *C. ulmi* from these remaining 2 trunks on September 12, 1941, were unsuccessful, the organism apparently having died out sometime between the 18th and 24th month of storage.

On September 23, 1941, 27 months after inoculation, samples were cut from 10 of the inoculated trees remaining. *Ceratostomella ulmi* was recovered from 9 of the 10 trees.

[1] Peehuman, L. L. A preliminary study of the biology of *Scolytus sulcatus* LeC., Jour. Econ. Ent. **31**: 537–543. 1938.

[2] Buchanan, W. D. *Scolytus sulcatus* LeC. transmits Dutch elm disease fungus under controlled conditions. Jour. Econ. Ent. **33**: 250–251. 1940.

In December, 1940, and in January, 1941, 107 samples of apple wood infested with *Scolytus sulcatus* were collected at 17 points in 4 counties in New Jersey and at 15 points in 3 counties in New York within 1,000 feet of where elms infected with *Ceratostomella ulmi* had been found and removed by the Bureau of Entomology and Plant Quarantine.[3] Attempts to isolate

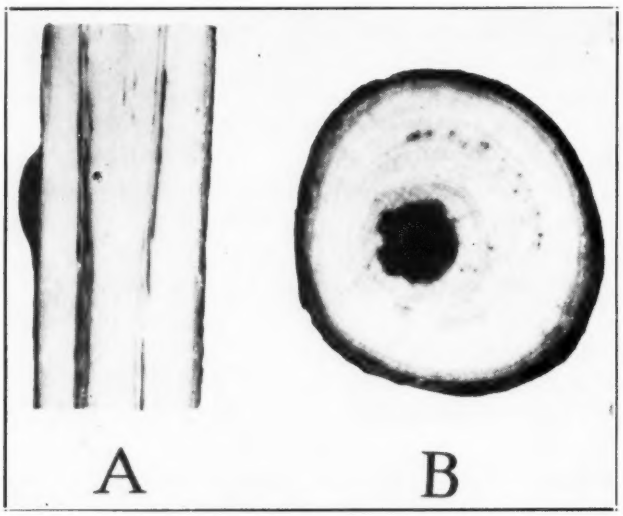

FIG. 1.—(A) Longitudinal section and (B) cross section of apple branch showing streaks of discoloration resulting from inoculation with *Ceratostomella ulmi*.

C. ulmi from representative maternal galleries from the 107 collections were made by the method described by J. M. Walter.[4] Of 535 none yielded *C. ulmi*.

The remaining infested material was then placed in rearing jars in the rearing room of the Division of Forest Insect Investigations, Morristown, N. J. The adult *Scolytus sulcatus* were collected regularly as they emerged, and were cultured. None of the 14,311 beetles from the infested material yielded *Ceratostomella ulmi*.

Even though *Ceratostomella ulmi* is capable of remaining viable for considerable periods of time in living apple trees and also in apple wood, the probability of *Scolytus sulcatus* transmitting the fungus to apple wood in nature and of the apple wood being an important source of inoculum for later spread, apparently is relatively unimportant at present in the Dutch elm disease control program.—S. J. SMUCKER, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, New Jersey.

[3] Acknowledgment is hereby made of the assistance rendered by J. F. Wootten and the various County Supervisors of the Dutch Elm Disease Control Office of the Bureau of Entomology and Plant Quarantine in making these collections possible. Acknowledgment is also made of the assistance rendered by W. D. Buchanan and Charles Griswold of the Division of Forest Insect Investigations in collecting apple wood infested with *Scolytus sulcatus*, and in rearing *S. sulcatus* from the infested material.

[4] Walter, J. M. Technique advantageous for the isolation of *Ceratostomella ulmi* from bark beetles. Phytopath. (Abstracts) **25**: 37–38. 1935.

BOOK REVIEW

THE AMERICAN SOCIETY OF AGRONOMY AND THE NATIONAL FERTILIZER ASSOCIATION. *Hunger Signs in Crops*, 340 pp., 95 figs., with 79 colored plates. $2.50. Judd & Detweiler and Standard Engraving Co. (Washington). 1941.

The publication of "Hunger Signs in Crops" makes available to plant pathologists a valuable reference for use in diagnosing plant diseases.

The book presents up-to-date information on the abnormal macroscopic conditions that are induced in agricultural plants when the supply of one, or more, of the food materials in the soil is insufficient. Since a deficiency of any one element may induce quite different symptoms on different plants, the plants are for the most part dealt with individually. Thus, there are chapters covering nutrient deficiencies in tobacco, corn and small grains, potato, cotton, truck crop plants, deciduous fruits, legumes, and citrus. The food materials dealt with are nitrogen, phosphorus, potassium, magnesium, calcium, boron, manganese, sulphur, iron, copper, and zinc. Each chapter has been written by specialists who have made extensive investigations on the crop concerned. Consequently, the description of the various deficiency symptoms are characterized by clarity of definition and a full appreciation of the complexities involved. At the end of each chapter a selected list of references is given. An outstanding value of the book is the large number of photographs in natural color illustrating the various disorders described in the text. There are 79 full-page color plates and many photographs in black and white. It is a text that should be on the shelf of every plant pathologist who contacts field problems, and who must make field diagnoses.—H. R. MCLARTY, Dominion Laboratory of Plant Pathology, Summerland, British Columbia.

NOTICES

SUMMER MEETING AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Summer Meeting of the American Phytopathological Society will be held June 25–26, 1942, in the Secor Hotel, Toledo, Ohio. The first session will begin at 10:00 a.m., June 25.

The purpose of this meeting is to assist in coordinating the various war emergency efforts of the Society. The functions, accomplishments, and future plans of the Society's War Emergency Committee, subcommittees, and sectional committees will be reported briefly with plenty of opportunity for discussion. Topics to be discussed, if time permits, include: fungicides —their availability and substitutes, coordinated research programs, duties of extension pathologists, draft deferment, disease surveys, quarantines, seed treatment and certification, cooperation with workers in other sciences, and other timely subjects.

A summer meeting of this nature is new for our Society, but presents an excellent opportunity to become intimately acquainted with the emergency programs and problems, and to contribute constructive criticisms and suggestions for future procedure.

When making hotel reservations indicate that you expect to attend the American Phytopathological Society Meetings.

COMMITTEE ON PROGRAM AND ARRANGEMENTS
1942 SUMMER MEETING

CONSERVATION OF SCHOLARLY JOURNALS

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited

supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

ANNOUNCEMENT

"In the July issue of *The Botanical Review* there will be an extensive review by Dr. W. J. Robbins and Dr. V. Kavanagh of the New York Botanical Garden and Columbia University on 'Vitamin Deficiencies of the Filamentous Fungi' which will be a tabulated summary of growth substance requirements of 375 species. Authors' reprints will not be distributed, but separate copies may be purchased for 50 cents each by pre-publication orders to *The Botanical Review*, New York Botanical Garden."

THE report of the Committee on Nomenclature and Classification of Plant Viruses having been, through error, omitted from the Annual Report of the Society, published in the April number of PHYTOPATHOLOGY, is here presented, together with the covering motion adopted by the Society.

The motion made, seconded, and adopted by the Council in session at Dallas, Texas, Dec. 28, 1941, and subsequently presented to the Society in regular business session, was, in substance as follows: The Council realizes that this committee has a very difficult assignment, and that it has been a hard-working committee, and, in view of the fact that it has not yet been possible to arrive at final recommendations, the Council recommends that the present reports be received with appreciation as reports of progress, and laid on the table. Following is the report of the committee:

REPORT OF THE COMMITTEE ON NOMENCLATURE AND CLASSIFICATION OF PLANT VIRUSES

The Committee approves (6 for; Gardner and Johnson against) the designation of plant viruses by a system of Latin binomials such as that used for scientific naming of organisms. An essentially similar system is used in naming the substances dealt with in Materia Medica. There is good ground for hope that the Committee will soon sponsor and recommend to the Society for approval and publication a simplified, compromise system of virus classification.

During the past year much progress has been made by some of the subcommittees selected to compile the synonyms and descriptions of viruses

associated with the plants of the principal crop divisions. Completion of the assignments will be hastened if the use of a Latin binomial as the preferred designation for a virus is generally accepted.

EUBANKS CARSNER, Chairman
C. W. BENNETT
M. W. GARDNER
F. O. HOLMES
JAMES JOHNSON
H. H. MCKINNEY
H. H. THORNBERRY
FREEMAN WEISS